The Abdus Salam
International Centre for Theoretical Physics

United Nations
Educational, Scientific and
Cultural Organization

IAEA
International Atomic Energy Agency

2038-23

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

1 - 5 June 2009

Single Molecule Studies of Homolog Pairing and Effects of Force on Molecular Structure and Function

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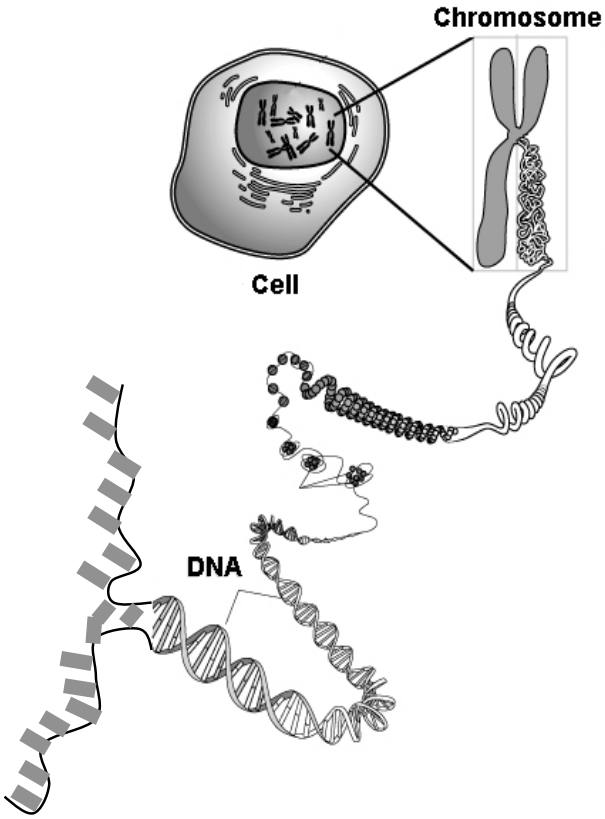
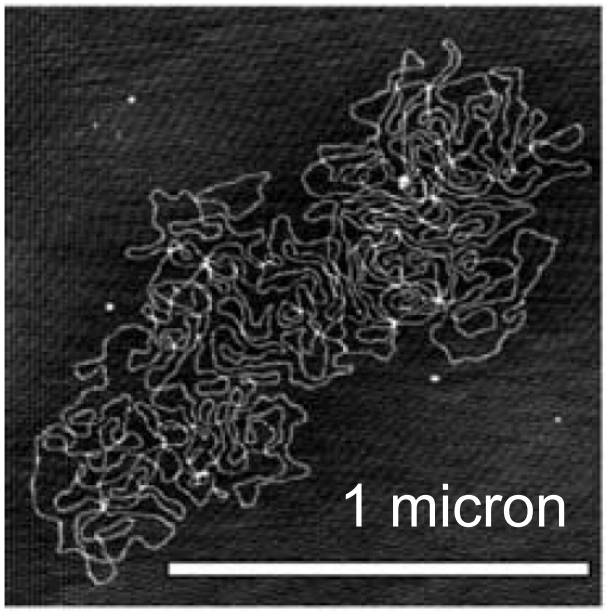
Single Molecule Studies of Homolog Pairing and Effects of Force on Molecular Structure and Function

(Sequence and Charge Matter: All Interactions are Local DNA Mechanical Properties Play a Crucial Role in RecA function)



http://www.koshlandsciencemuseum.org/exhibitdna/intro02.jsp

AFM of of 100,000 bp dsDNA



dsDNA persistence length
~ 50 nm ~ 140 bp

S. Araki et al. / Chemical Physics Letters 418 (2006) 255–259

C. Bouzigues, Buddhapriya Chakrabarti, V. Coljee, R. Conroy, C. Danilowicz , M. Dowlatshahi , K. Hatch, K.Kim, Y. Kafri, Kleckner, C. Lee, C. Limouse, J. B. Lucks, D. R. Nelson, M. Prentiss, C. Brian Roland, E. Shahknovich, H. Stone, J. Vlassakis J. Vlasic, J. D. Weeks, G. Whitesides, J. Williams, A. Zhou

Biologists Don't Appreciate Biophysics Experiment more than Biophysics Theory

- Biophysicists make wonderfully precise measurements of wholly uninteresting quantities
- I am not interested in physicists explaining something I already know, I want them to tell me something I don't know that will save me bench time

Outline

- Sequence Dependent dsDNA Pairing
  - Overview
  - Single molecule pairing experiment in a buffer with no proteins and only monovalent salts
  - Questions from Biologists
  - Newer Experimental results
  - Not why, but why not!
  - A new tool for self-assembly
- Stretching double stranded DNA Creates New Conformations (Sequence and Charge Matter and some base sequences are magic)
  - Monovalent salts do not have interchangeable screening
  - Changing the anion also changes the screening
  - The conformation of overstretched DNA depends on the ends to which the force is applied
    - » Force selectively flips “magic” bases at 65 pN but no more bases open until 100 pN
    - » Temperature selectively flips “magic” bases at 37 C, but no more bases flip or denature until T>68C
- DNA and RecA
  - Different force induced conformations interact differently with RecA
  - Some base sequences are magic for RecA
    - » force allows them to immediately nucleate RecA
      - < 100 such sites in 48,500 bp lambda
    - » Pre-melting to 37C and then quenching allows immediate nucleation of RecA at room temperature
  - We propose a new model where almost all of the function of RecA is based on the mechanical properties of DNA

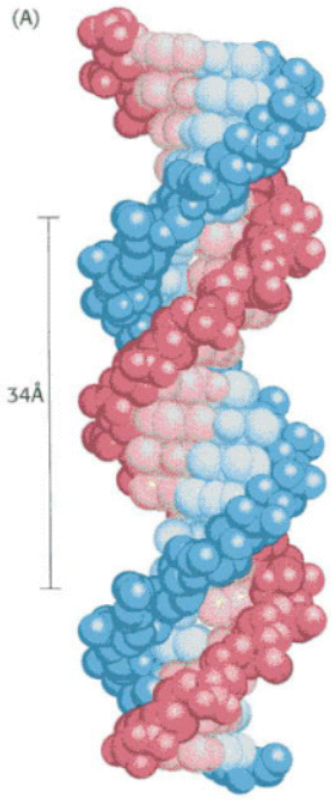
A Great Mystery of Life

- **Why do two like coiled polymers with the same charge come together and align in perfect registration?**

DNA Dimensions

DNA is usually double-stranded

Dimensions of B DNA (the common form)
- 35.4 Å repeat
- 3.4 Å / base
- 10.4 bases per helix turn
- Diameter is 23.7 Å
- Right-handed screw sense



dsDNA persistence length
~ 50 nm ~ 140 bp
ssDNA persistence length
~ 1-2.5 nm ~ 3-10 bp

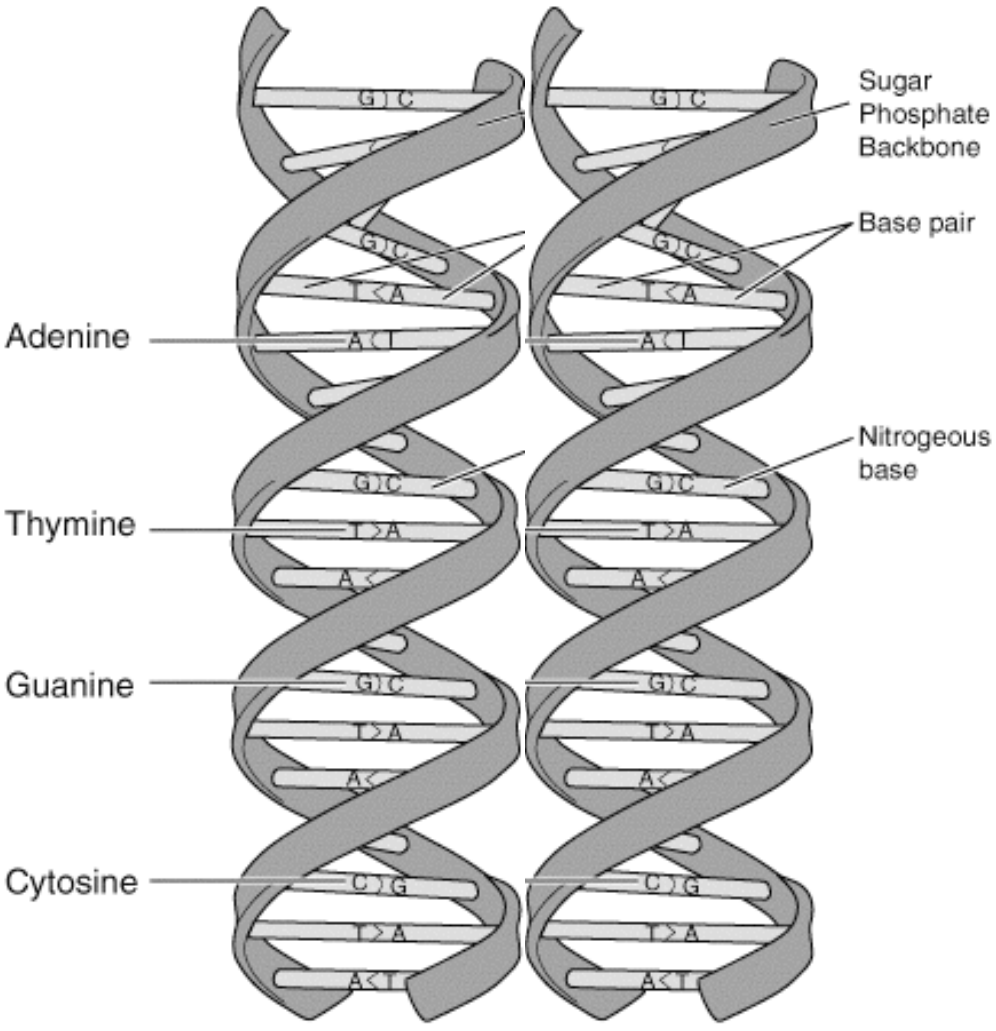
Human genome ≈ 3.3 x 10^9 bp
≈ 25,000 genes

Highly Charged

E= 5 x 10^7 V/m 4 nm from the DNA

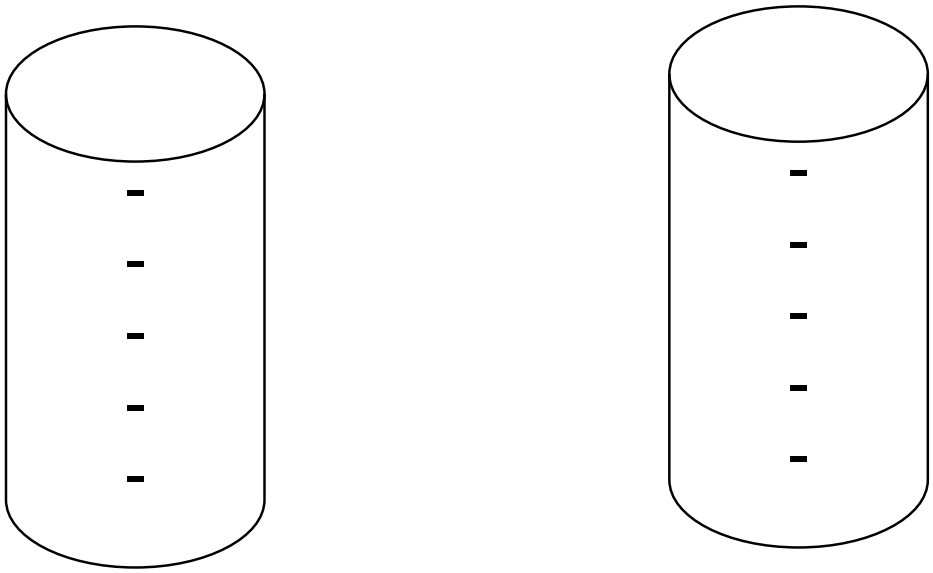
Interactions Between Double Stranded DNA Molecules

- In vivo chromosomes pair correctly
- In vitro, multiple dsDNA molecules group together
  - DNA aggregates in multivalent buffers at low concentration (microg/mL)
  - At concentrations 1000x higher (mg/ml) DNA aggregates at room temperature in monovalent buffers and high pressure



Simplest Picture Fails

- **Why do objects with the same charge attract?**

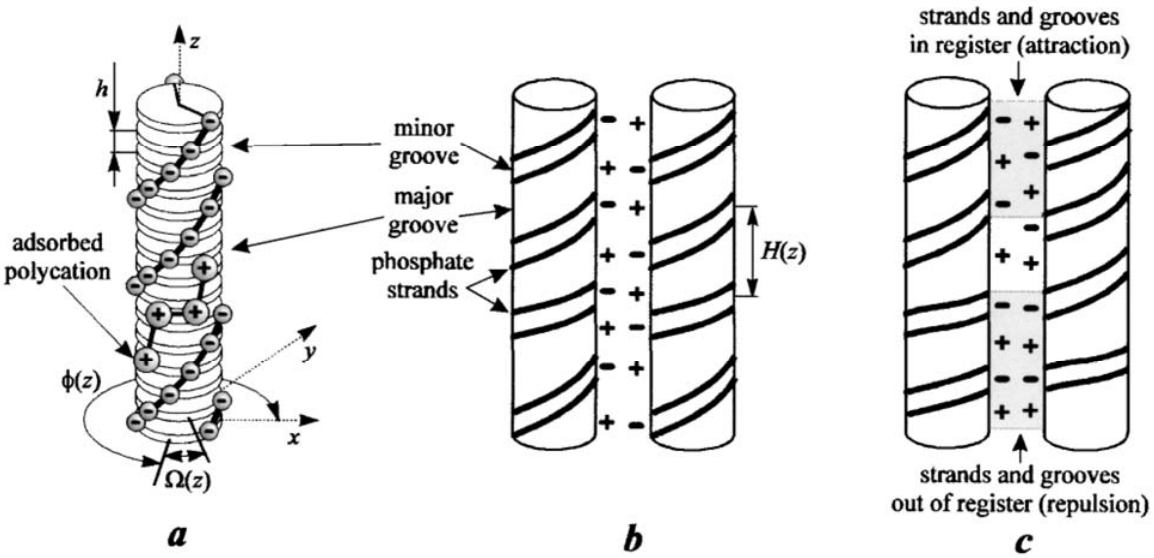


- **Counterions just screen removing all interaction**

Must Include Spatial Dependence

Sequence Recognition in the Pairing of DNA Duplexes

A.A.Kornyshev and S.Leikin, *Phys.Rev.Lett.* 2001, **86**, 3666.



(a) *B*-DNA schematically drawn as a stack of base pairs (disks). Each base pair has two negatively charged phosphate groups.

The homology recognition well as an innate property of DNA structure, A.A. Kornyshev, A. Wynveen, PNAS,Vol 106 Number 12, pages 4683-4688, 2009

Helical coherence of DNA in crystals and solution Wynveen A, Lee DJ, Kornyshev AA, et al. NUCLEIC ACIDS RESEARCH Volume: 36 Issue: 17 Pages: 5540-5551 2008

Structure and interactions of biological helices Kornyshev AA, Lee DJ, Leikin S, et al. Source: REVIEWS OF MODERN PHYSICS Volume: 79 Issue: 3 Pages: 943-996 2007

Torsional Deformation of Double Helix in Interaction and aggregation of DNA. A.G.Cherstvy, A.A.Kornyshev, and S.Leikin, *J.Phys.Chem.B,* 2004, **108,** 6508.

Nonlinear effects in torsional adjustment of interacting DNA. A.Kornyshev and A.Wynveen,. *Phys.Rev.E,* 2004, **69,** #041905, 1-14.

DNA-DNA interaction beyond the ground state. D.J.Lee, A.Wynveen, and A.A.Kornyshev, *Phys. Rev. E,* 2004, **70,** #051913, 1-12.

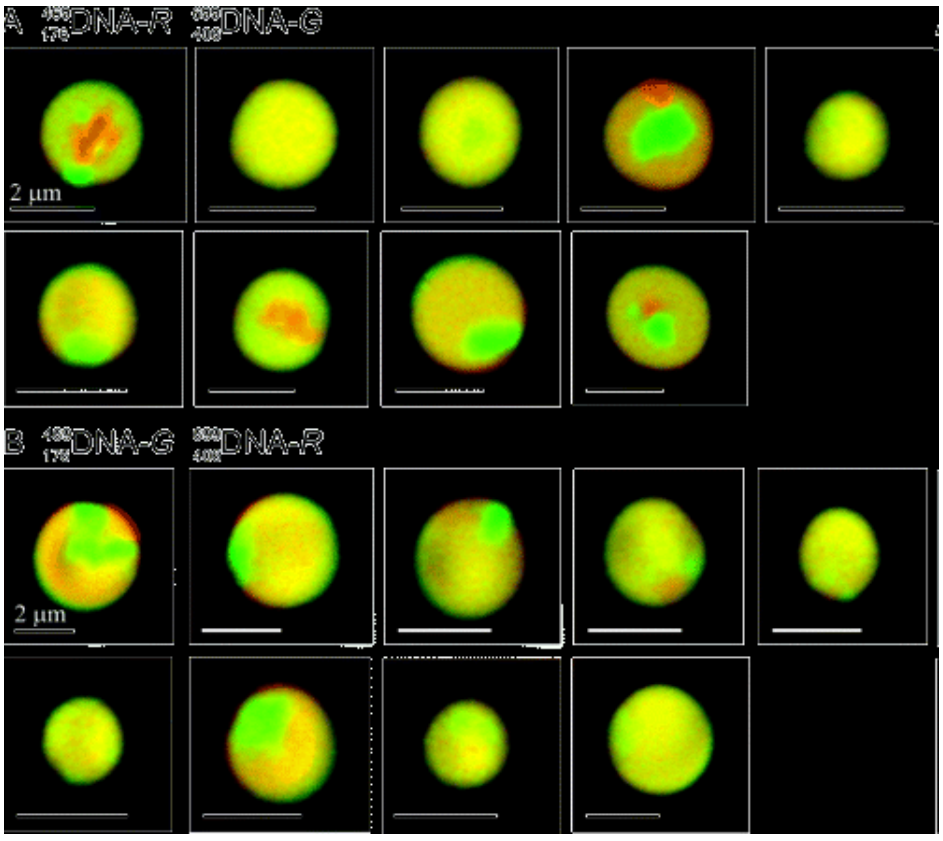
Counterion-induced Attraction Between Rigid Polyelectrolytes Nielsgrønbech-jensen,robertj.Mashl,robijnf.Bruinsma,and William M.Gelbart PRL Vol 78, Number12 1997.

Sequence Dependent Pairing Does occurs in Vitro without Proteins

DNA Double Helices Recognize Mutual Sequence Homology in a Protein Free Environment

Geoff S. Baldwin,* Nicholas J. Brooks, Rebecca E. Robson, Aaron Wynveen, Arach Goldar, Sergey Leikin,* John M. Seddon,* and Alexei A. Kornyshev*

J. Phys. Chem. B, **112 (4), 1060 -1064, 2008.**



- "fragments with identical sequences were approximately 2 times more likely to be found near each other than the fragments with different sequences. Such segregation suggests that the pair interaction between the same fragments is more favorable than the interaction between fragments with different sequences by ~1 *kT* (0.6 kcal/mol)"

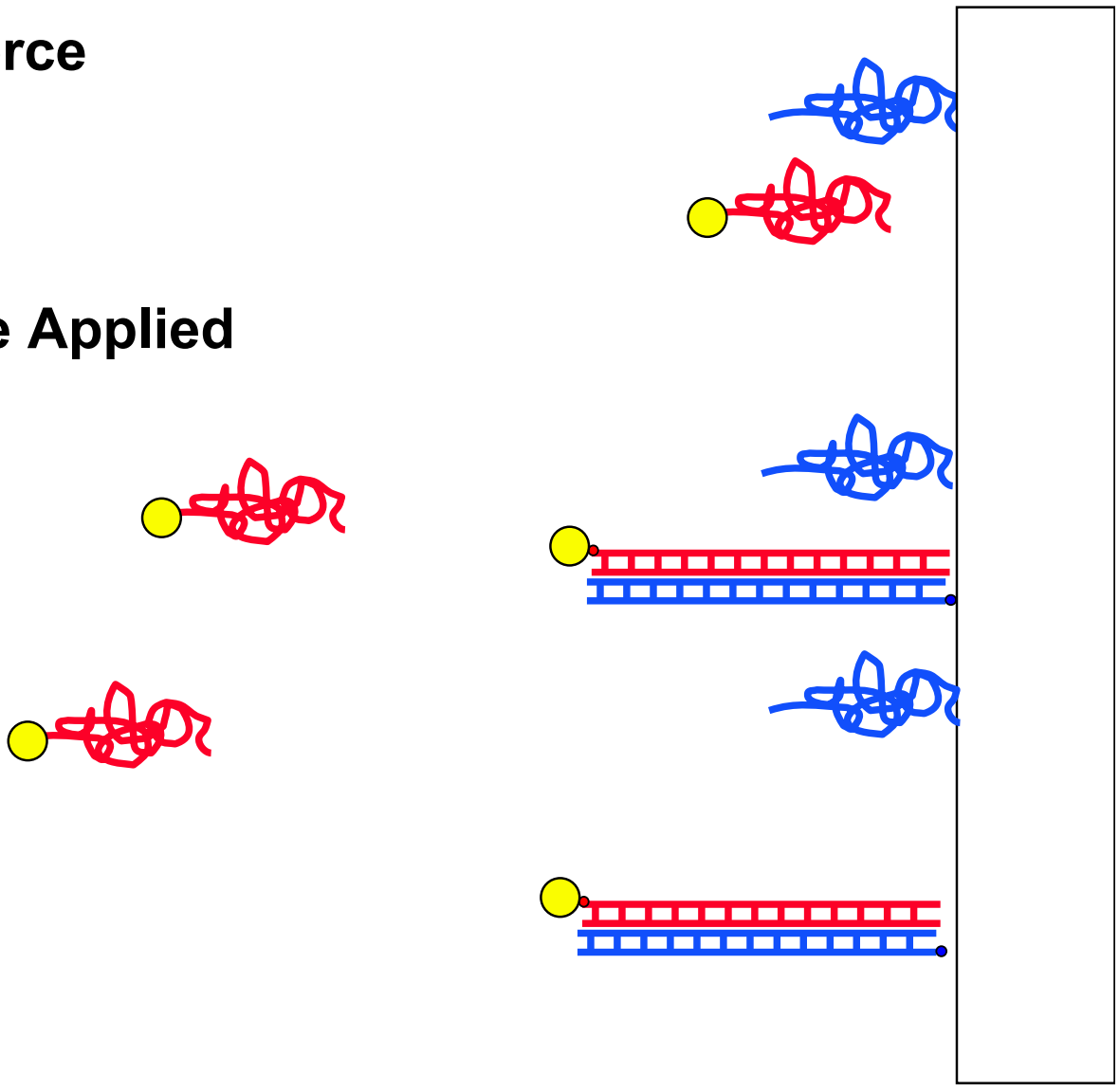
Single Molecule dsDNA Pairing Detection

Red DNA sticks only to the capillary due tp dig/anti-dig bond

Green DNA sticks only to the bead due to biotin streptavidin bond

No force

Force Applied

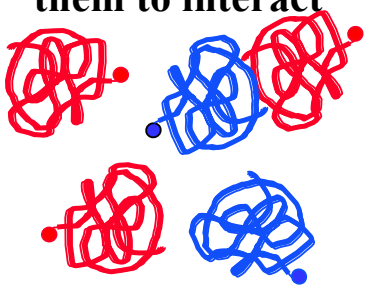


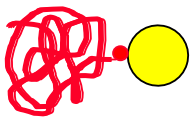
stc: 10

Single Molecule dsDNA Pairing Experiment

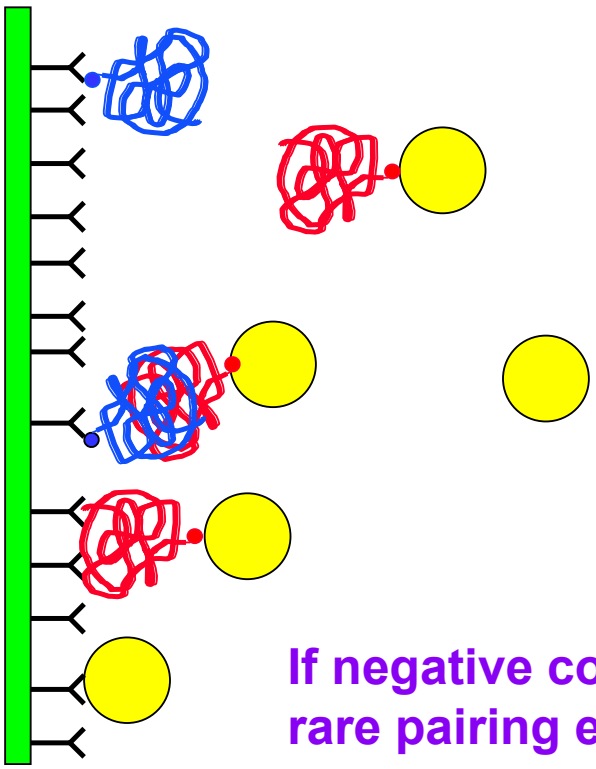
No proteins, no crowding agents, only monovalent salts

Step 1 Mix 2 types of DNA in a tube and allow them to interact

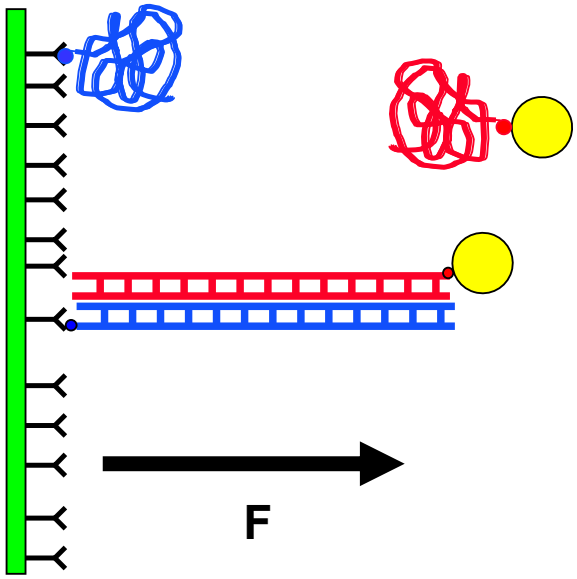




Step 2 Add beads and insert into the capillary



Step 3 Apply a magnetic wash and measure the beads that remain bound -> bound by paired dsDNA

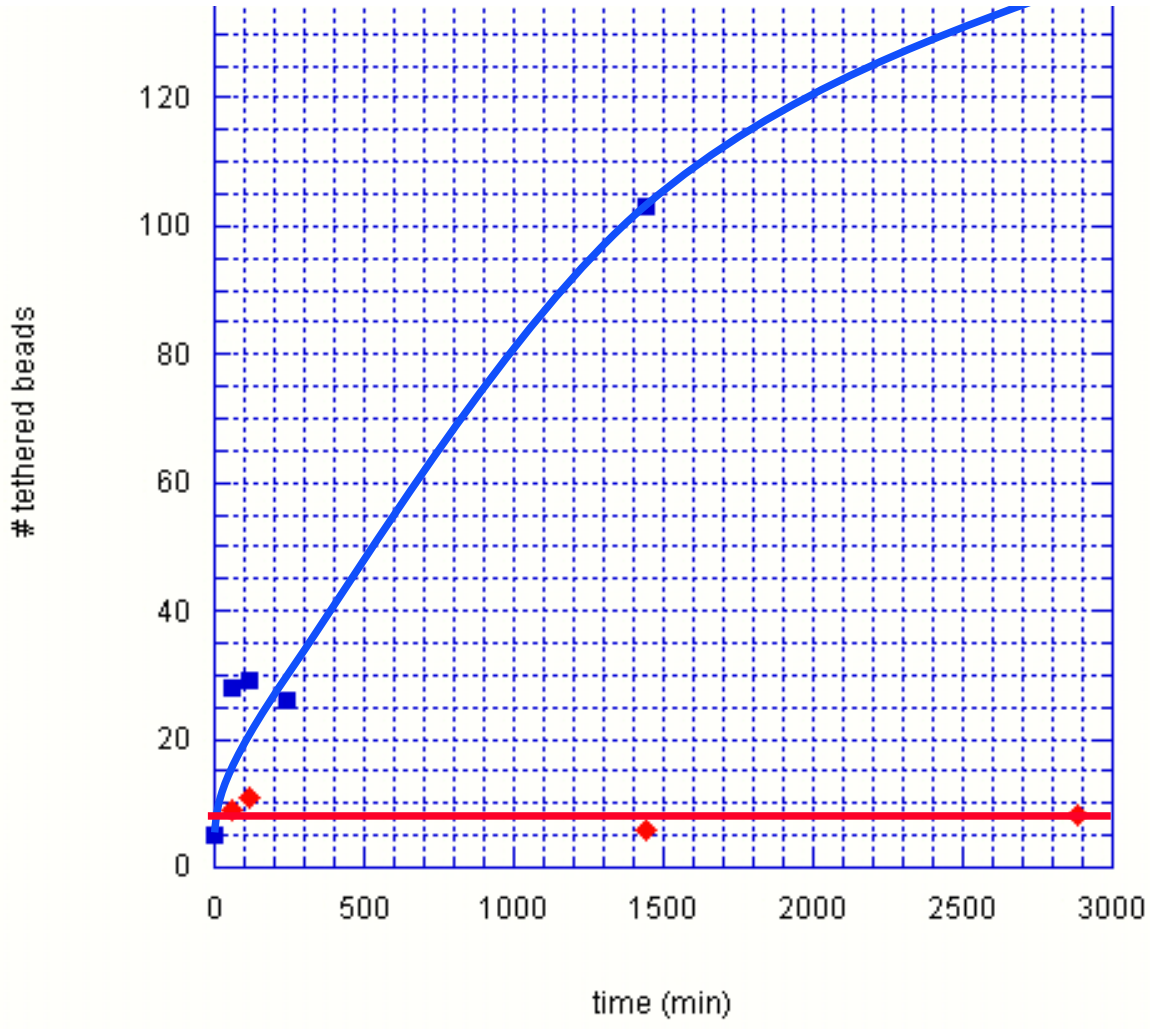


If negative controls are good, very rare pairing events are easily detected

It is Sequence Dependent!
(bar code)

Matched 5k bp of Lambda matching with Complete Lambda vs time

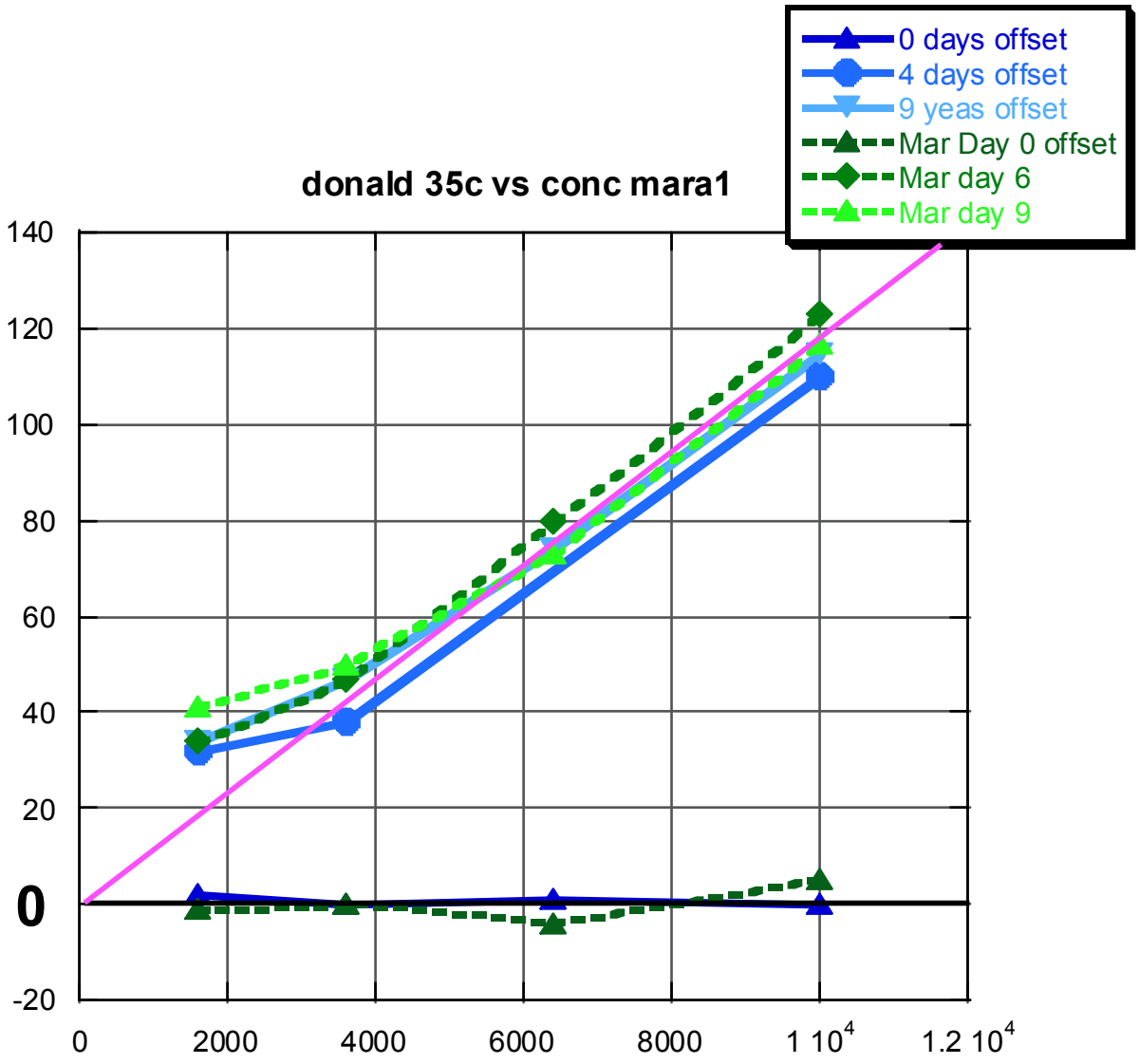
Unmatched PCDN3 5k bp matching with Complete Lambda vs time



It is a Pair Process

Aggregation vs Concentration Squared

Various waiting times

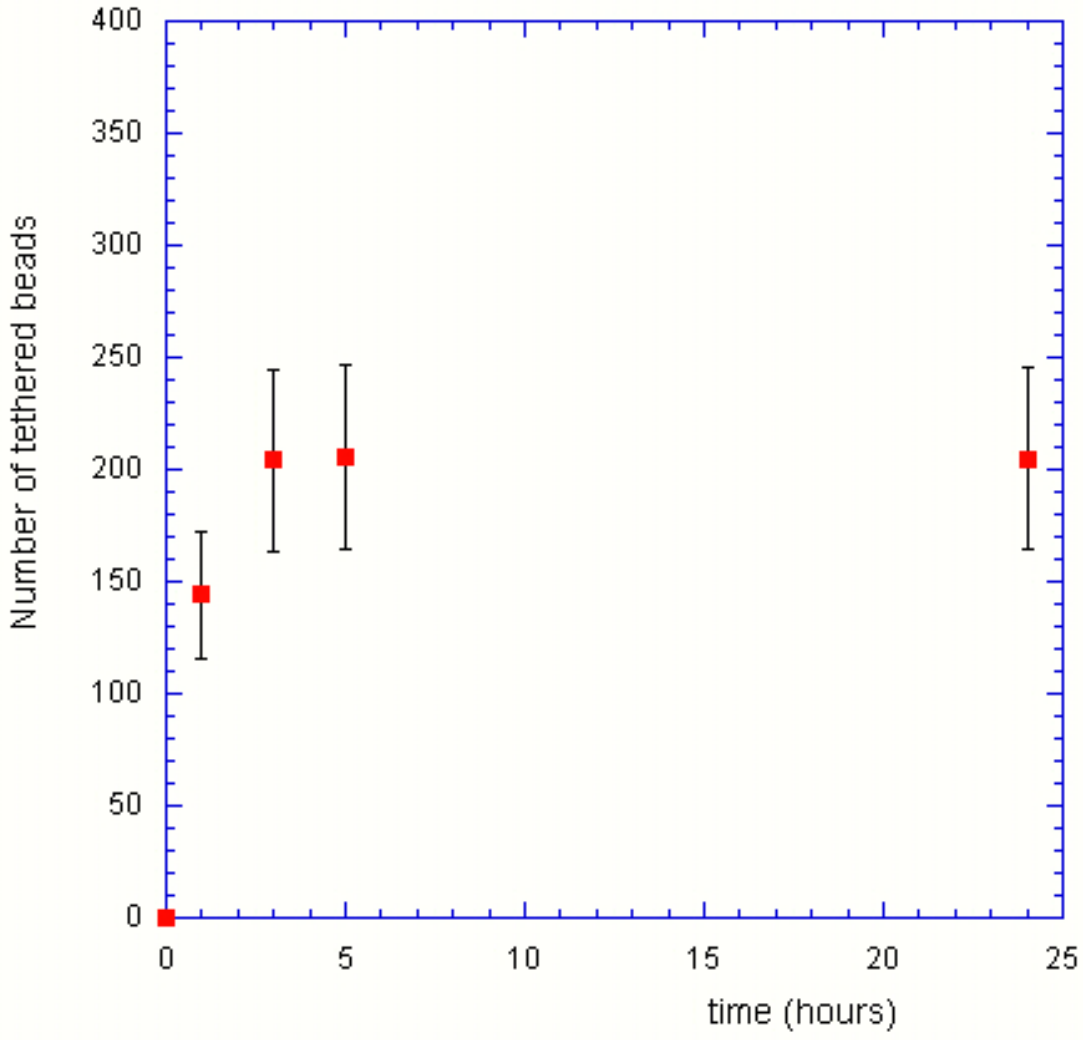


(micrograms/mL)²

(in vivo concentration 100 mg/mL)

stc: 13

Pairing Occurs Before the Beads are Added

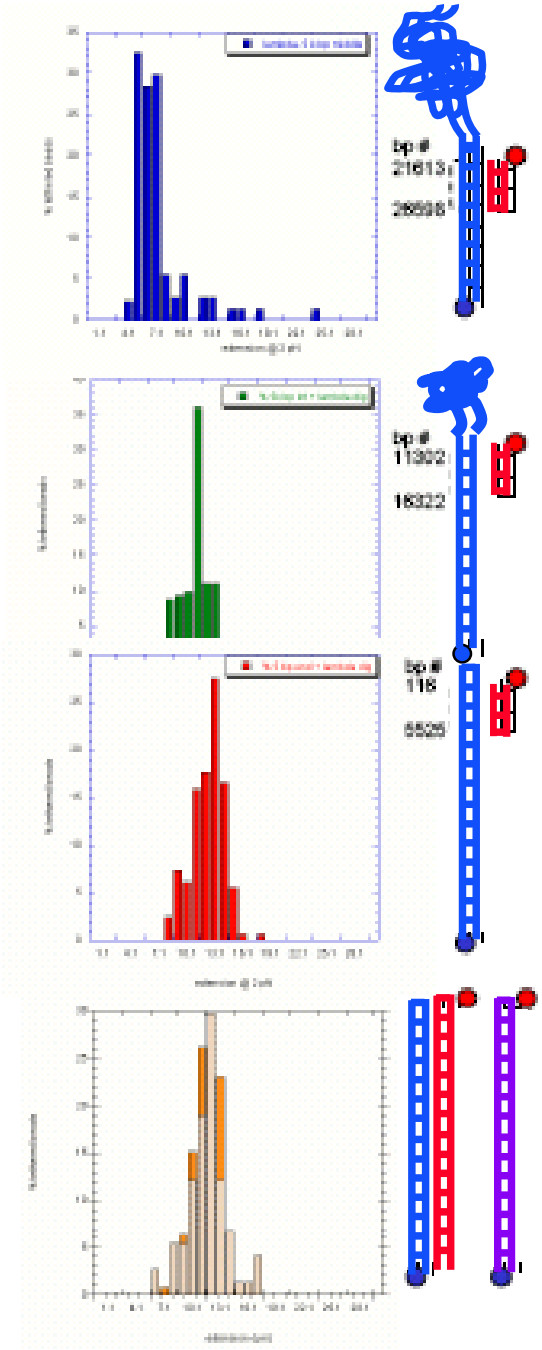


Saturation is due to gel formation?

Experiments with Part of the Lambda Sequence

- Previous slides used two complete matching lambda phage, so the final bead position is the same as for one lambda bound at both ends
- Test sequence dependence using shorter partial sequences where the beads should bind at the ends of the short sequences which do not occur at the full length of lambda phage
  - More stringent test of sequence dependence
  - Additional support for pairing vs aggregation

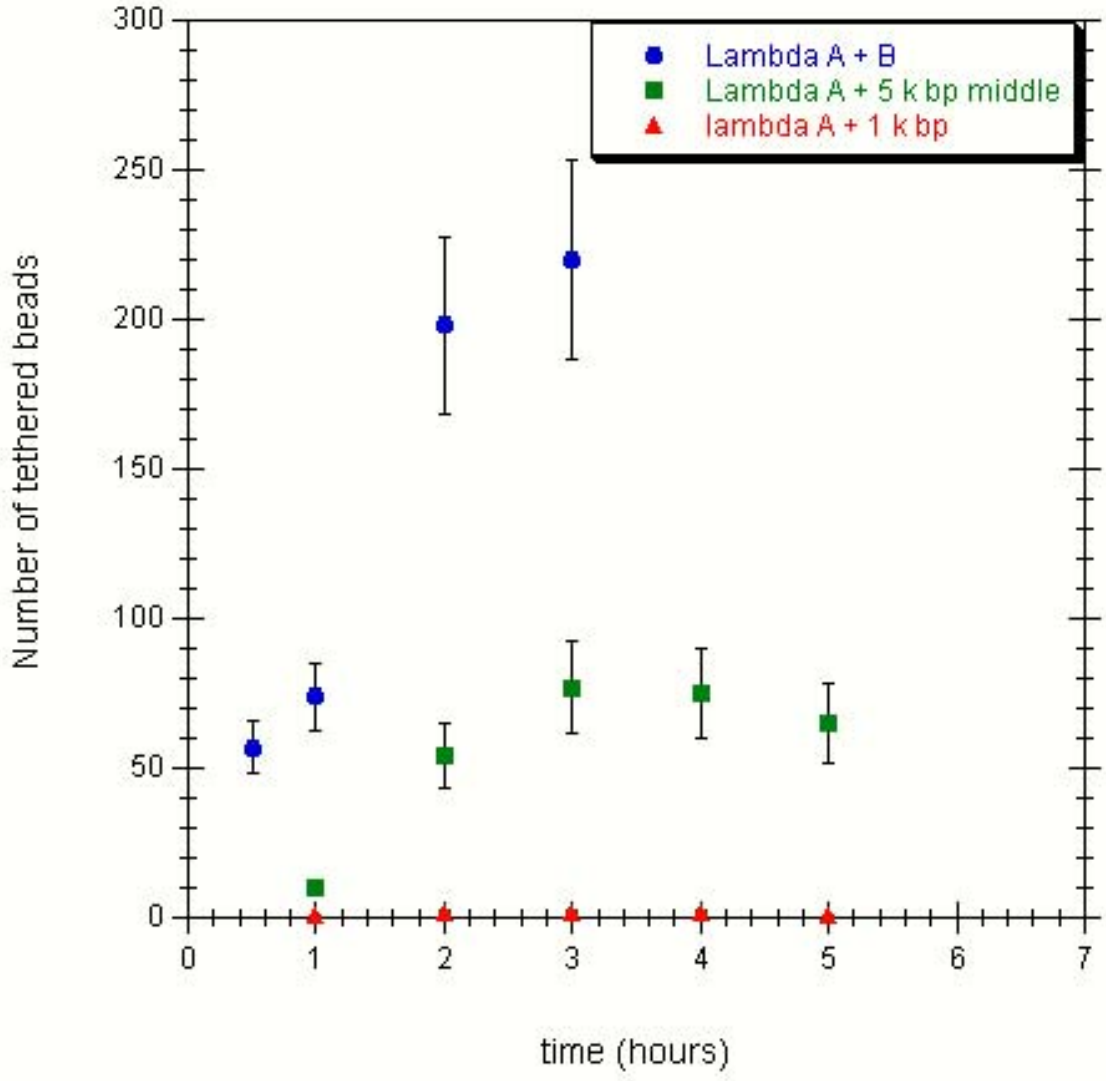
Spatial Position of 5k Fragments against Complete Lambda Indicates Sequence Dependence



The cartoons at the right show the position of the sequence match for the three different 5k fragments against complete lambda
The histograms show measured bead distances from the surface for each case

Comparison between Complete Lambda matched with Lambda and one single Lambda linked at both ends

Dependence of Binding Success of the Length of the Partial Lambda Sequence

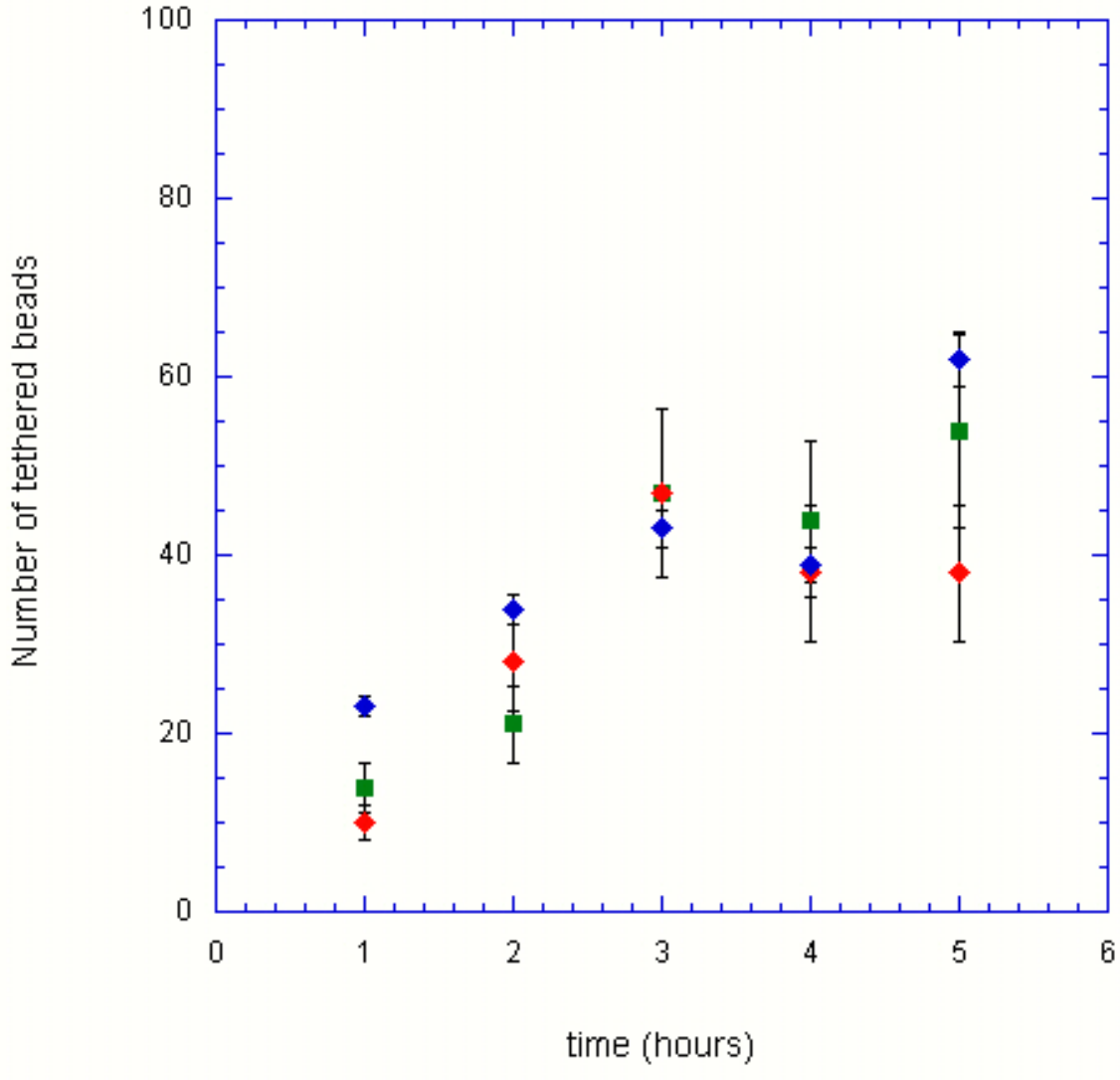


Success rate decreases with length

Probably search success rather than binding strength

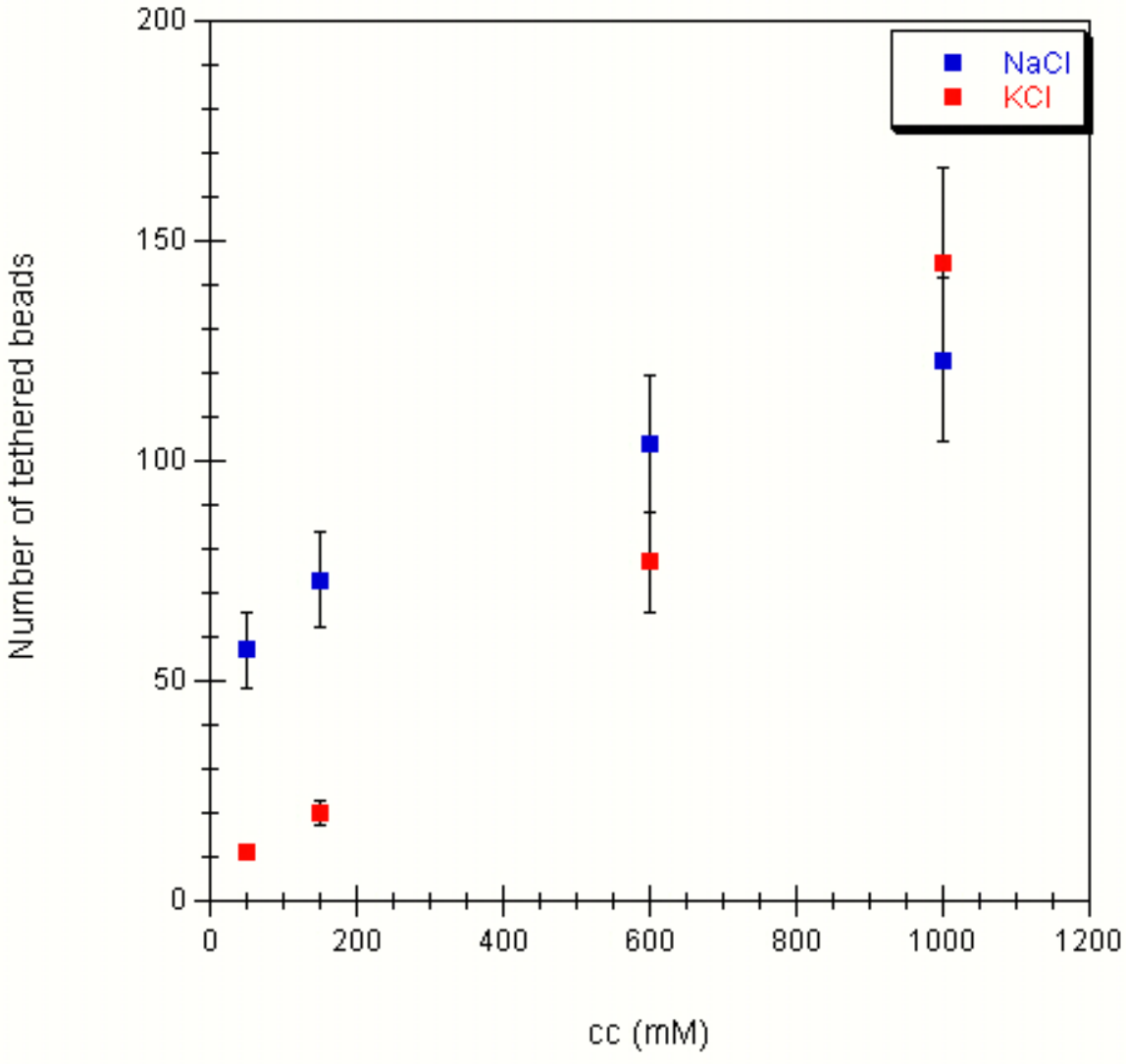
Insensitive to Competitors

60 mg/ml of biotinylated 5 kb and digoxigenin labeled lambda phage DNA
With PBS and 50 mg/ml human genomic DNA
With 0.1 % m/v bovine serum albumin (BSA)
Also did PCDNA3 and random hexamers



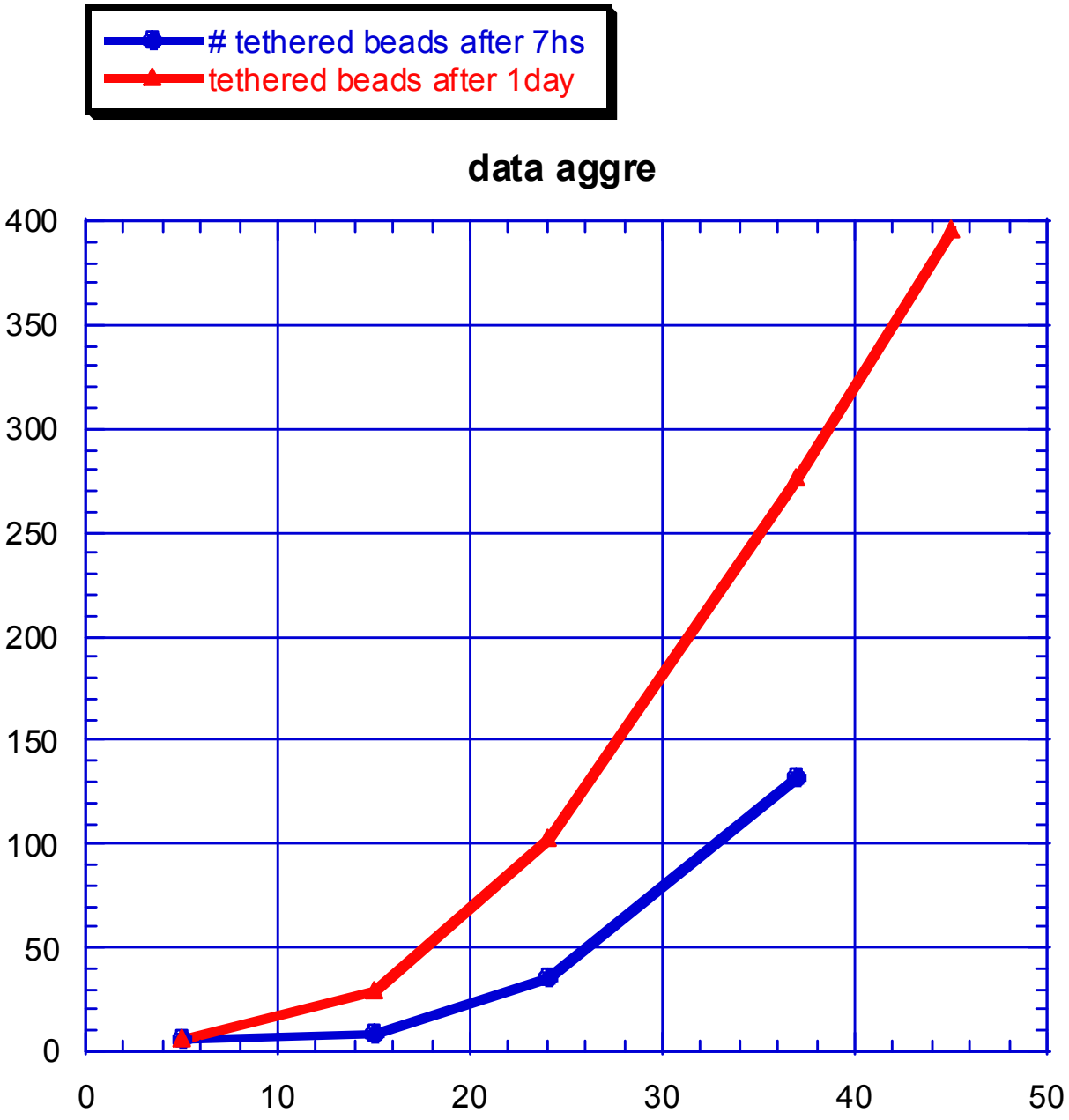
Intermolecular pairing between 5 kb base pair fragments and lambda phage molecules in the presence of non specific competitors. Samples containing 60 mg/ml of biotinylated 5 kb and digoxigenin labeled lambda phage DNA in PBS (green), PBS and 50 mg/ml human genomic dsDNA (red), PBS and 0.1 % m/v bovine serum albumin (BSA) (blue).

Monovalent Salt Dependent



Pairing Increases with screening

Aggregation vs Temperature



Some Predictions of Temperature Dependence are not in Trivial Agreement

Volume78, Number12 Physical Review Letters 24 March 1997 Counterion-induced Attraction Between Rigid Polyelectrolytes
Nielsgrønbech-jensen,robertj.Mashl,robijnf.Bruinsma,and William M.Gelbart

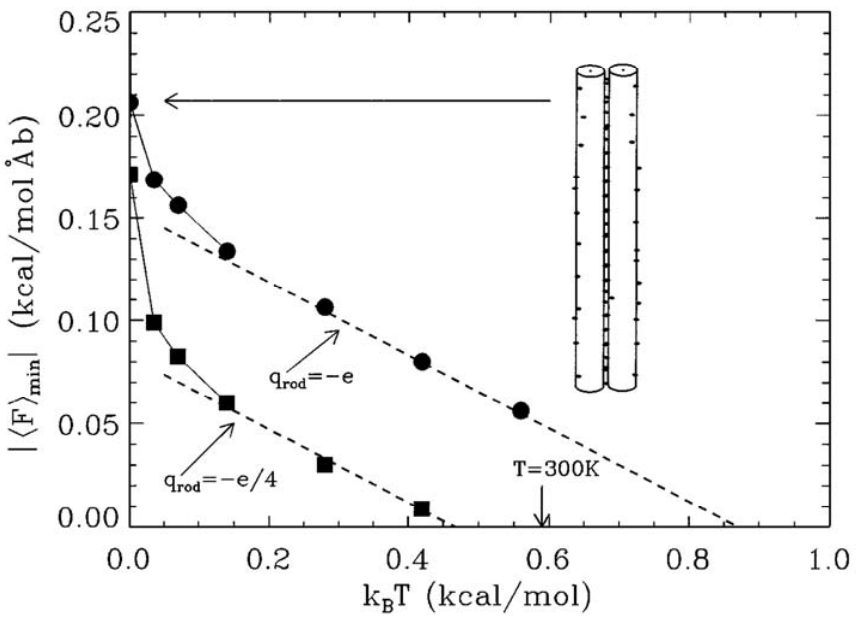


FIG. 3. Dependence of the maximum attractive force $|\langle F_{\min}\rangle|$ (per rod charge q_{rod}) between the rods, as a function of temperature T, for $q_{\text{rod}} = -e$ (top curve) and $q_{\text{rod}} = -e/4$ (bottom curve). The error in the data is comparable to the size of the circles and squares indicating the results. Inset: Counterion arrangement in the low-temperature limit for $q_{\text{rod}} = -e$. (Some counterions are obscured by the rods and are not shown here.)

Additional effects may provide agreement:

1. **bulk dielectric constant of water decreases with temperature changing sign of U/kT scaling, though bulk constant may not apply**
2. **fluctuations increase with T, which may result in improved pairing**

Additional Information

- PEG increases Binding
- Histones remove pairing of lambda (collaboration with Nicole Francis)
- 10-20 pN shear force consistent with recent calculations (A. Kornyshev Private Communication)
- Most Single Molecule data is ~ 5 years old

Biologist's Issues

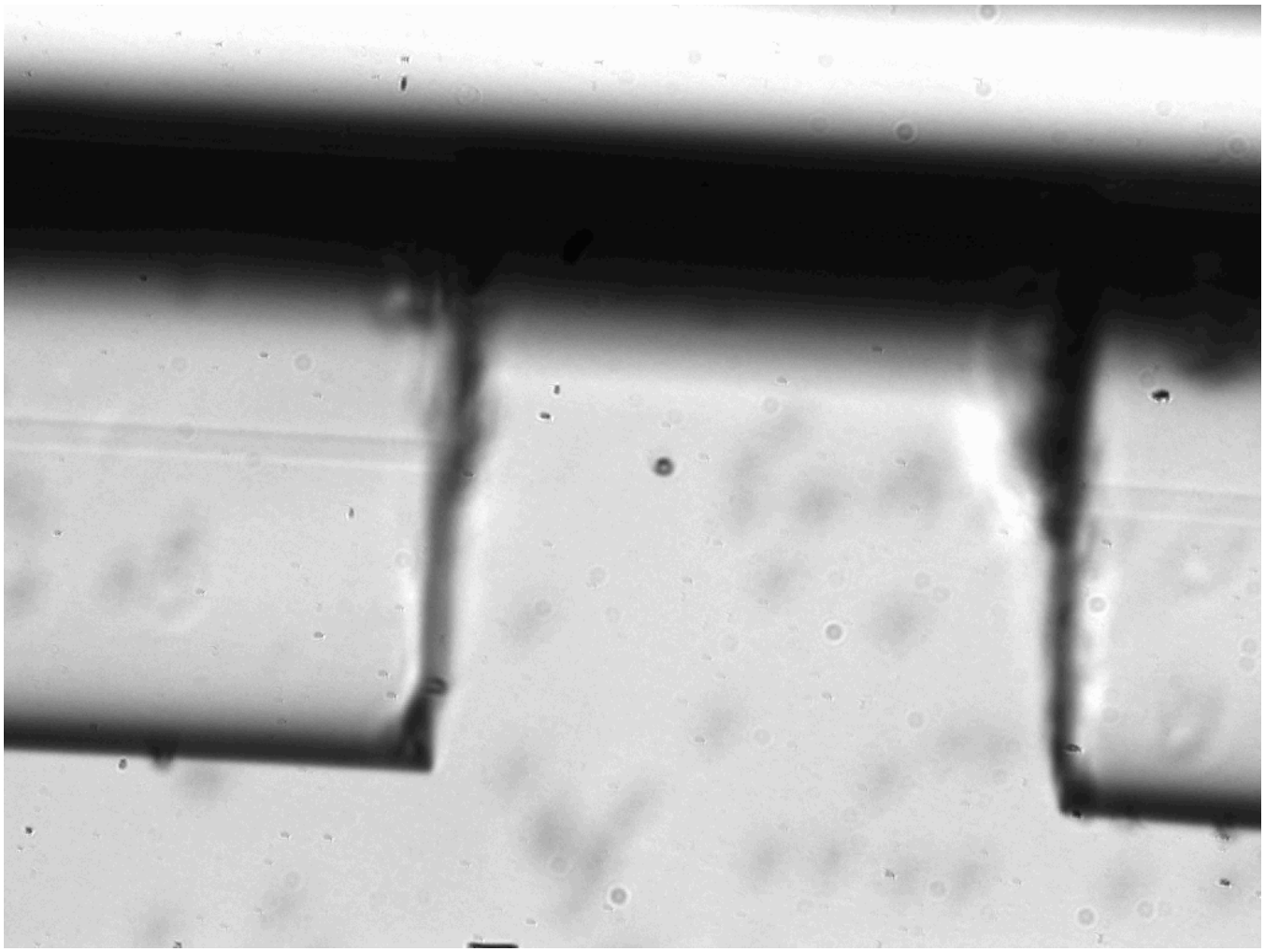
- **Issues with previous non-single molecule studies**
  - Sequence dependent aggregation is not sequence dependent pairing because clumping does not imply pairs of sequence with good registration
  - Some past experiments reported by physicists may have been Watson and Crick in Disguise
    - » Many previous biophysics experiments had open ends, bubbles, dyes that change dsDNA structure and create nicks, and/or PCR products
- **Issues with our single molecule work**
  - Temperature dependence is suggests melting
  - Why does the pairing saturate at < 10 % of the sample?
  - The presence of histones eliminates pairing for lambda phage

New Experiments to Address Issues Raised by Biologists

- **Watson-Crick issues**
  - Did experiments with 12 bp sticky ends and saw no increase in pairing even with available Watson and Crick
  - Did experiments with dsDNA heated to higher temps and then cooled to deliberately create bubbles and saw no increase in pairing
  - New dsDNA created in bacteria with 5 kb of lambda + 2.4 kb of other bacterial DNA on the ends and paired with lambda so there is no match in the open ends where sample was grown in bacteria not made directly in PCR
    - » Still shows pairing
  - Circle on circle does not show pairing possibly due to knots
- **Saturation at less than 100% pairing**
  - Optical tweezer shows very dense gel forms
  - Macroscopic rheometer also shows a very dense gel (collaboration with the Weitz lab)
  - Interesting implications for materials science: the resulting gel is MUCH stiffer at low concentrations than most known gels.

New Tools

- **Dual beam fiber-optic tweezer**



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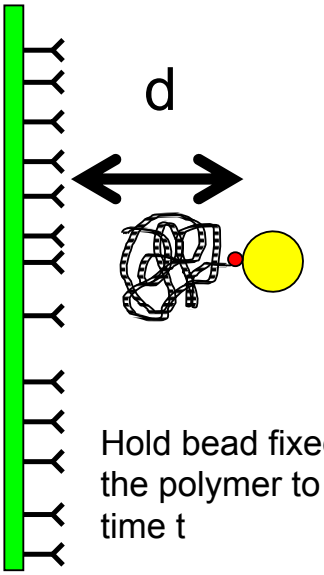
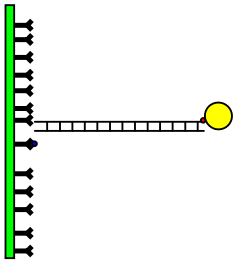
Dynamic Measurements of Binding for 1 Single dsDNA molecule



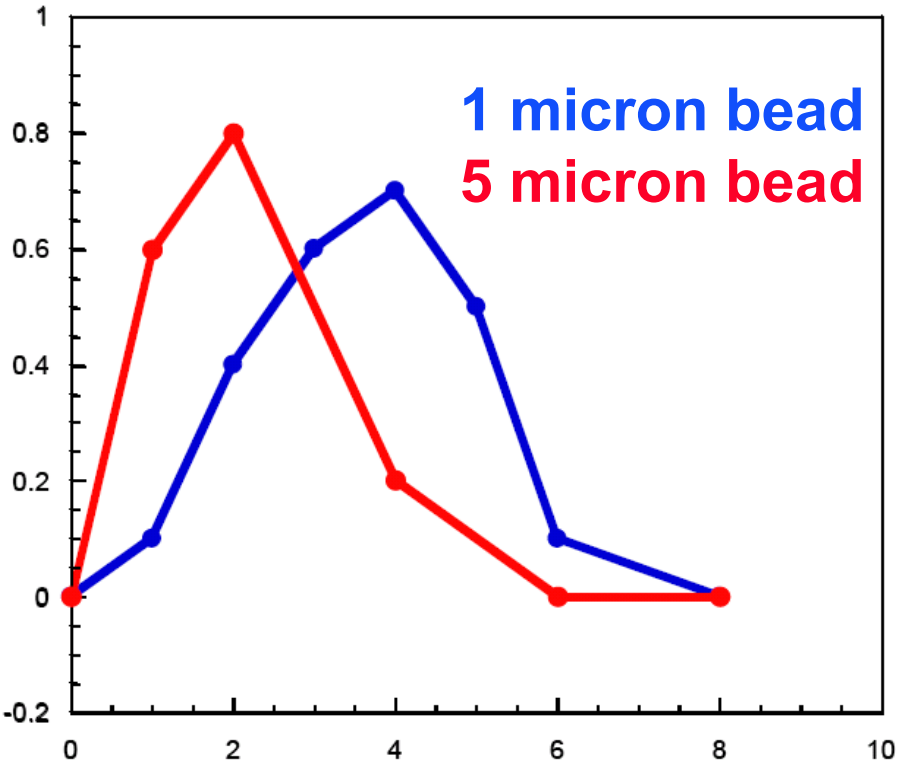
Hold bead fixed and allow the polymer to evolve for a time t



Bound

Test for binding

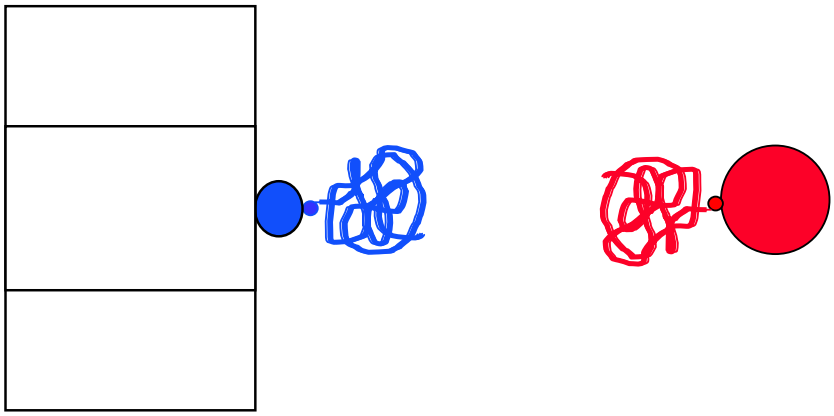
Unbound



S. Tyle

Experiments in Progress on Sequence Dependent Pairing of 2 Isolated Single Molecules

- **Sequence Dependent pairing using "fuzzy beads" and no DNA free in solution**
- **Repeated binding and unbinding possible**



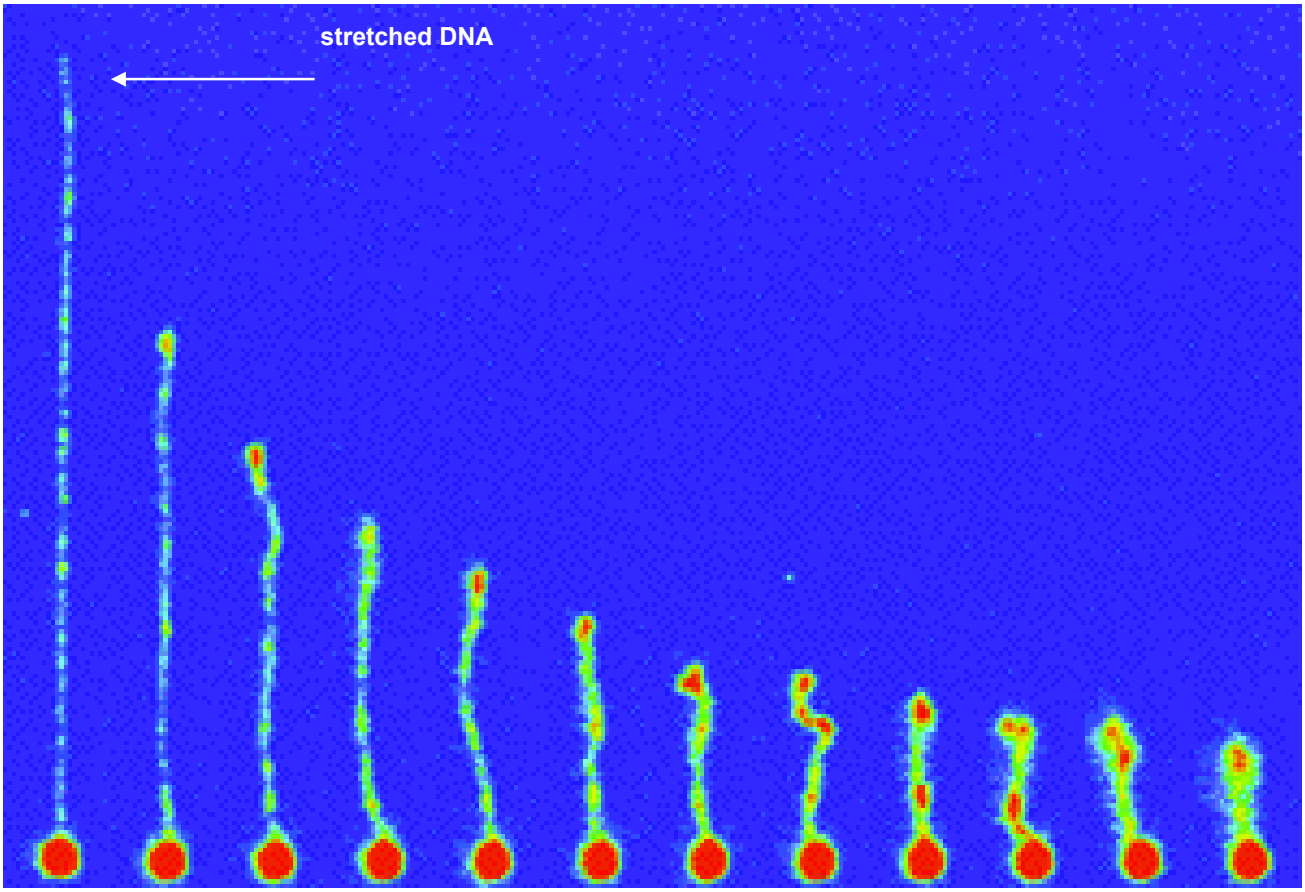
Not Why, but Why Not?

- **False Minima are a problem**
  - **General problem of self-assembly of long bar codes is that the energy error for one mistake scales as 1/N ->0 as n->infinity**
- **One solution is flexibility**
  - **Short rigid sequences with flexible linkers**
  - **Long linkers with fluctuating spacings as discussed already in the conference**

Additional Issues

- **Why does the pairing not occur all of the time?**
- **“ Matching” Chromosomes do not have identical sequences so even the “correct” pairing is wrong**

Force Induced Structural Changes in dsDNA



Time evolution of a single DNA strand stretched by tweezers and then released

(Steven Chu, Stanford University)

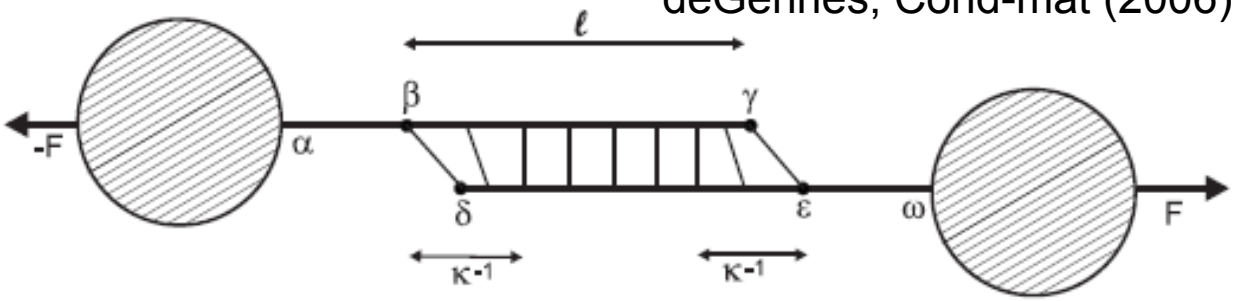
Biologists' Issue

- So?
- One interesting question is the mechanical stability of DNA with single strand breaks or pairs of single strand breaks

deGennes Predicted Shear force for 3'3' and 5'5' Pulling

- **For infinitely stiff backbone force shear force is evenly distributed among all base pairs -> F_{shear} α N**
- **Finite backbone stiffness redistributes force between backbones, -> long separations between single strand breaks are no more stable than short ones**

deGennes, Cond-mat (2006).



$$F_c = 2f_1 \left\{ \varkappa^{-1} \tanh\left(\varkappa \frac{\ell}{2}\right) + 1 \right\}$$

f_1 = rupture force for single base pair

l = number of base pairs

X^{-1} = adjustment length (in bps)

Low force limit f1 L

~ number of bases in sequence x binding force/bp

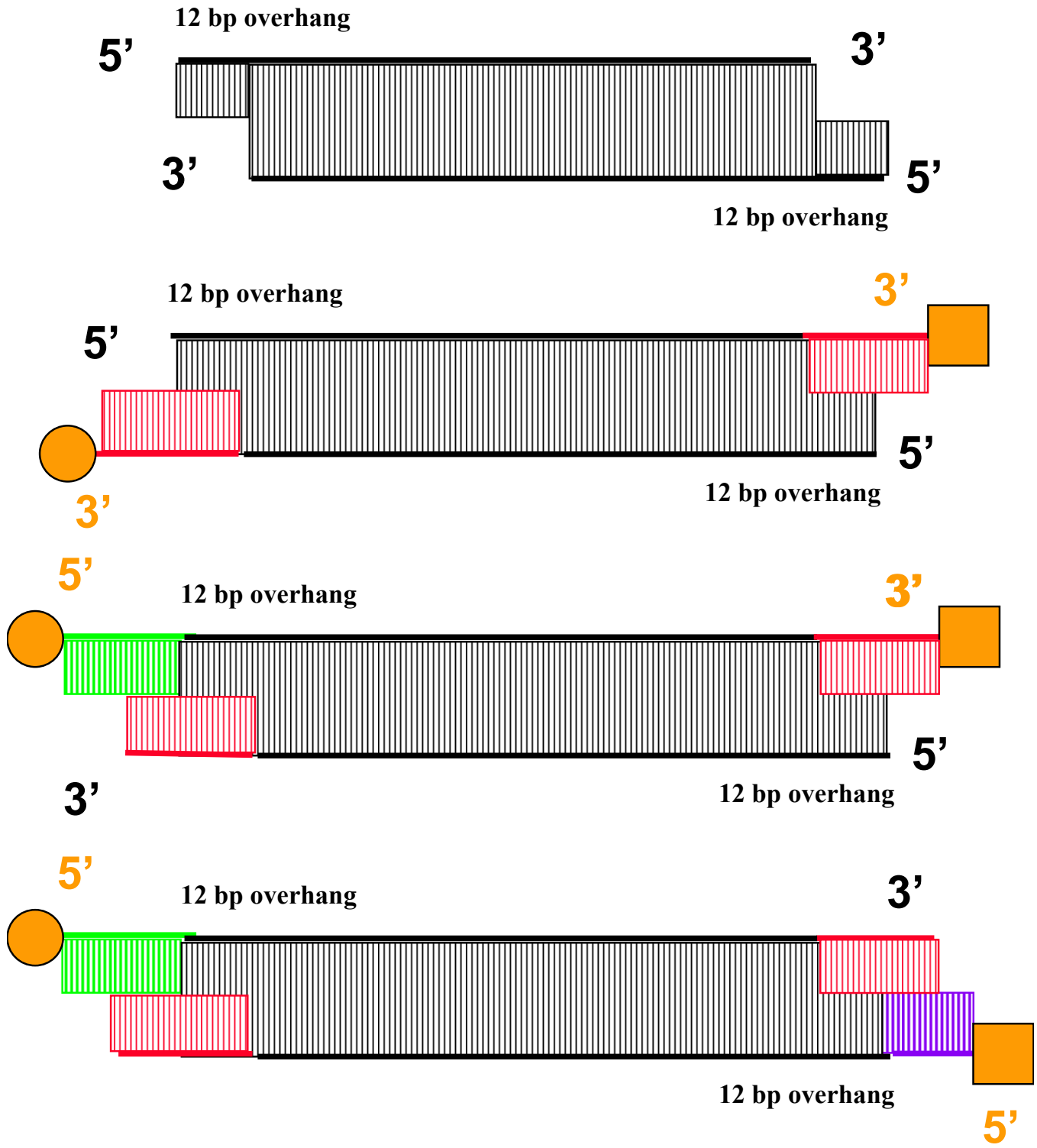
High force limit -> 2 f_1 χ^{-1}

~ number of base pairs over which force is distributed x binding force/bp

Theory allows the experiment to provide a ratio of the phosphate spring constant to the basepair spring constant

Lambda Phage

(48,502 bp)



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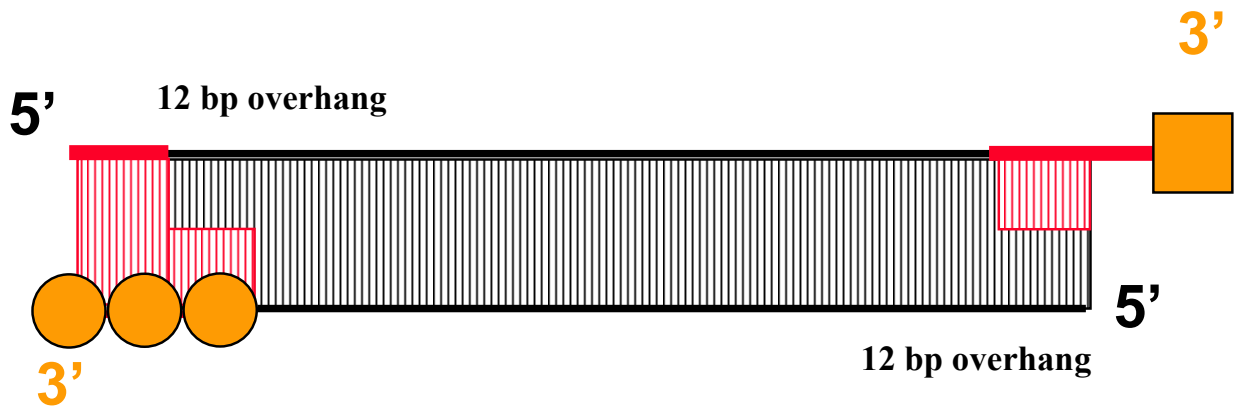
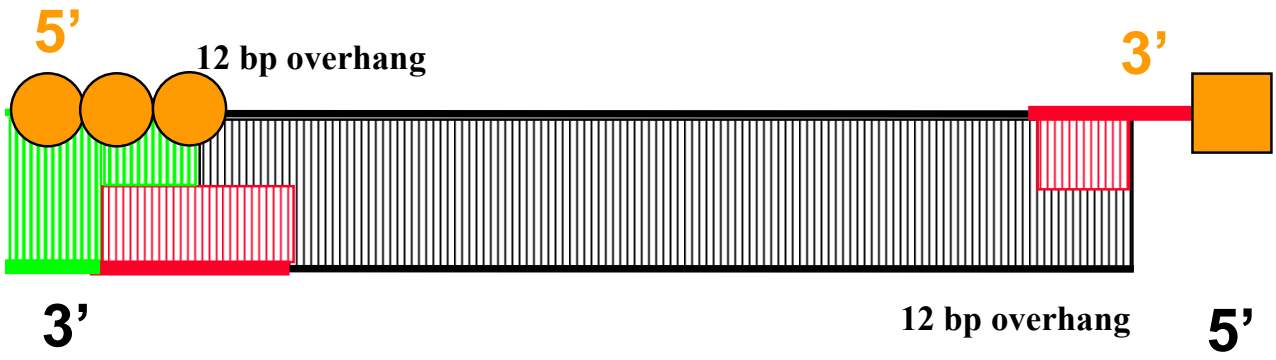
Previous Work





Force distribution over the phosphate strands is unclear

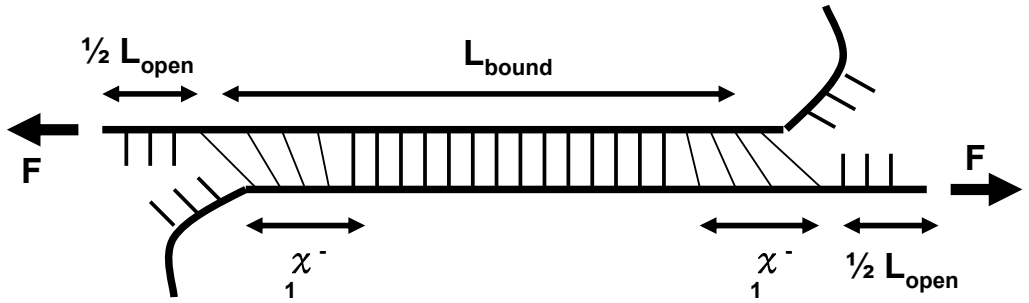
No 5’5’ at constant force

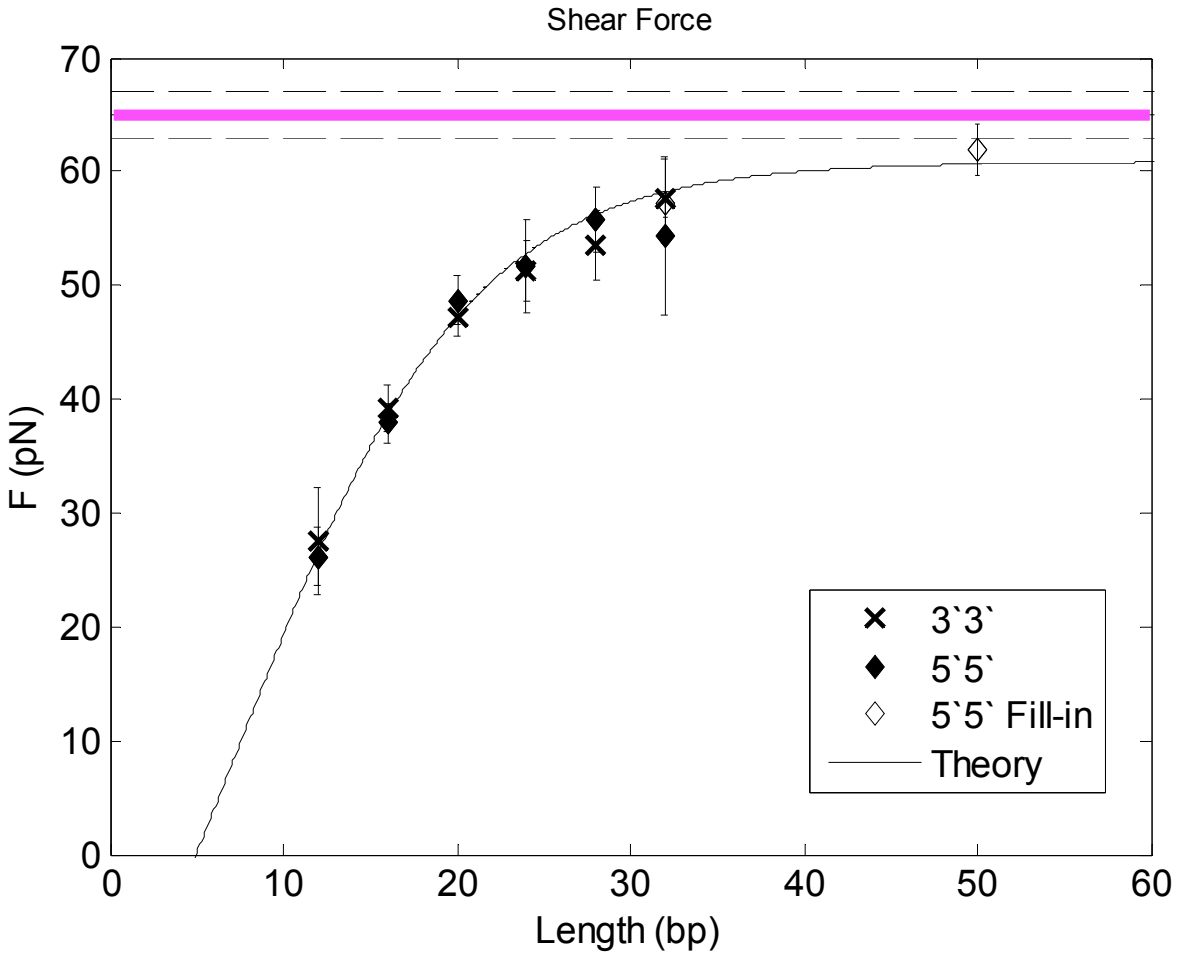
No Ligation?

stc: 34

Results for Short Sequences 3'3' and 5'5' show Same Shear Force

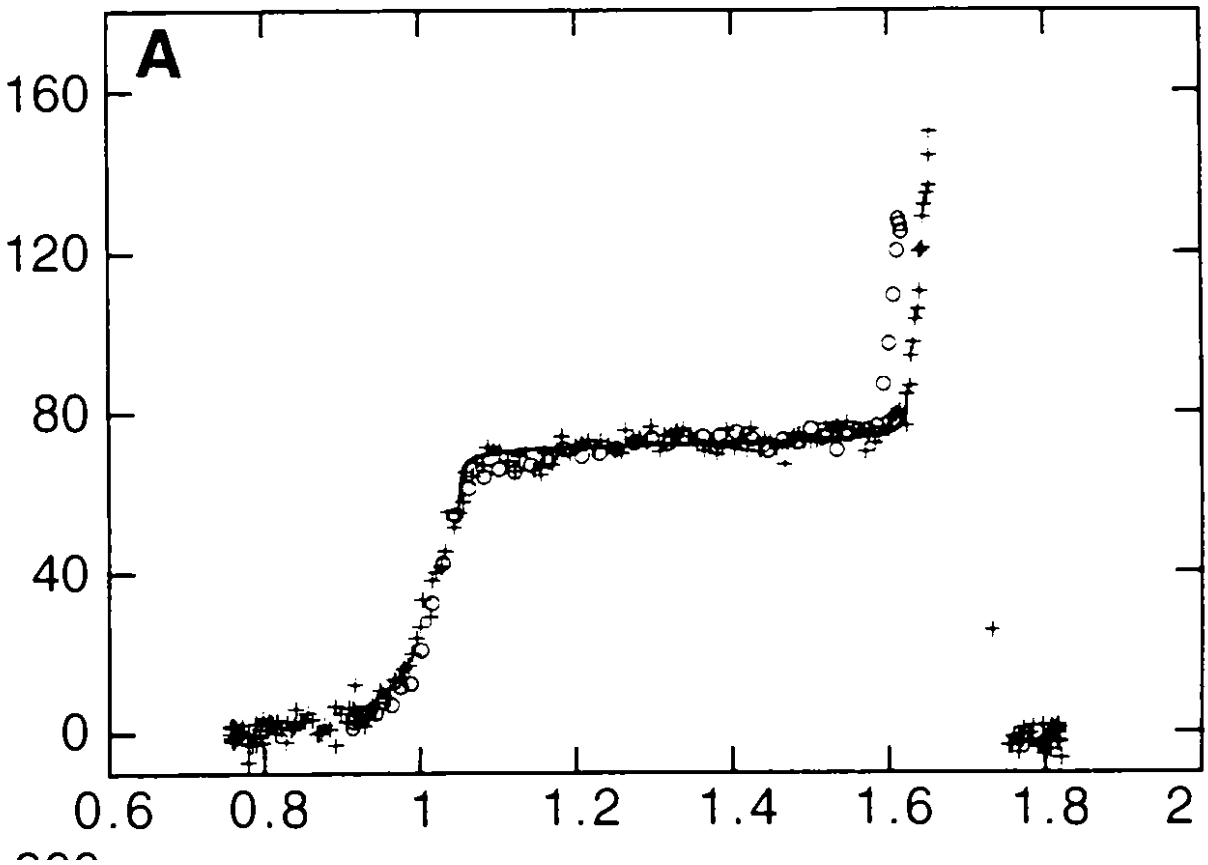
deGennes length of<7 bp





f1 = 3.9 pN, *Lopen* = 7 bp, and *c-1* = 6.8 bp. This value of *c* corresponds to a ratio *Q/R* = 92.5.

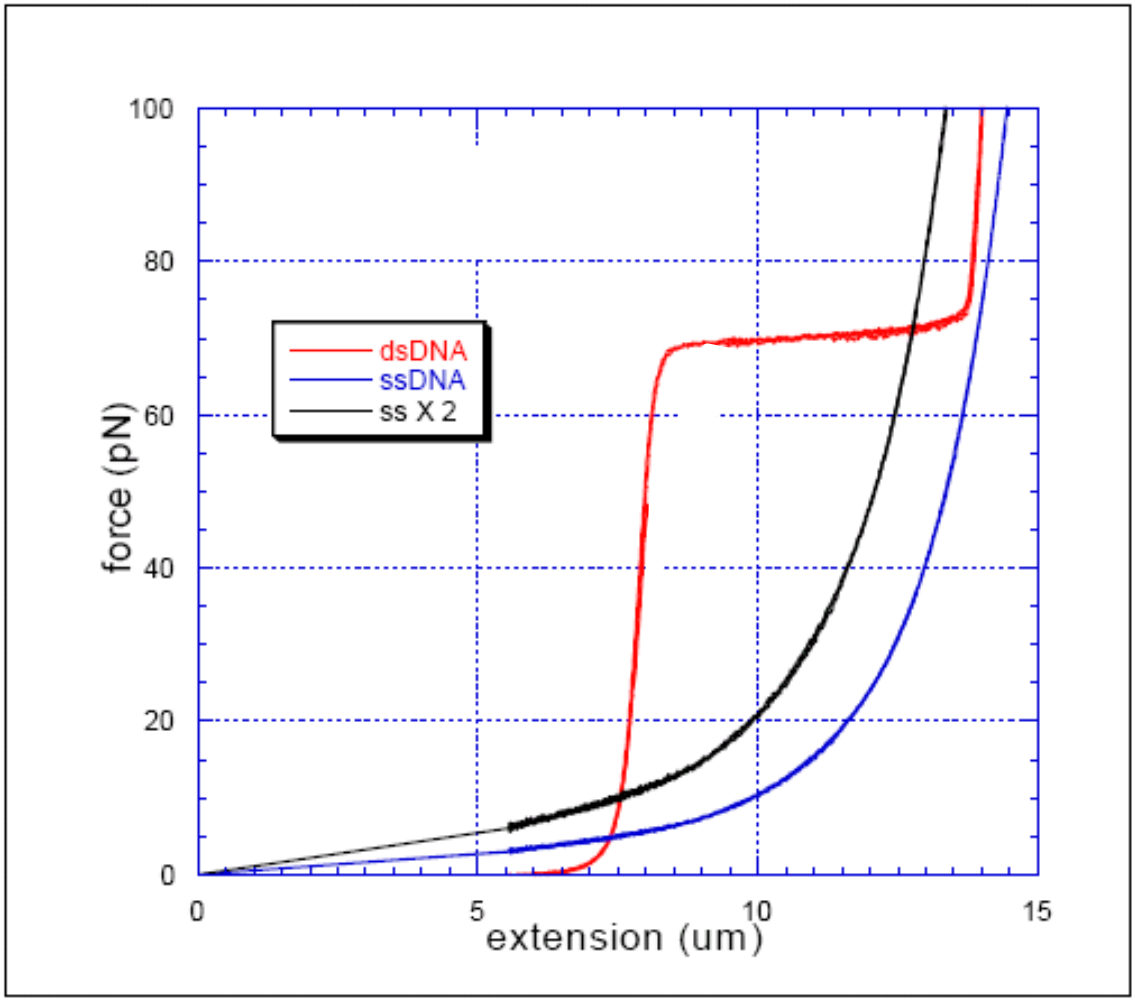
The Overstretching Transition



- **Rec A does this?**

Science, Vol 271 Feb 9 1998 p. 276

Data From S. Smith/Bustamante group



Note the slope of the measured double strand curve above the overstretching force matches the slope below the transition. Also, the measured slope for 1 ssDNA is much less steep than the measured slope for dsDNA and the slope for 2 ssDNA is also less than for ssDNA. Thus no linear combination of 1 and 2 ssDNA could reproduce the measured slope of the curve above the overstretching transition.

More Bustamante Data

Polymerization and mechanical properties of single
RecA–DNA filaments
MARTIN HEGNER†‡, STEVEN B. SMITH†, AND CARLOS BUSTAMANTE†§¶**PNAS** Vol. 96, pp. 10109–10114, August 1999

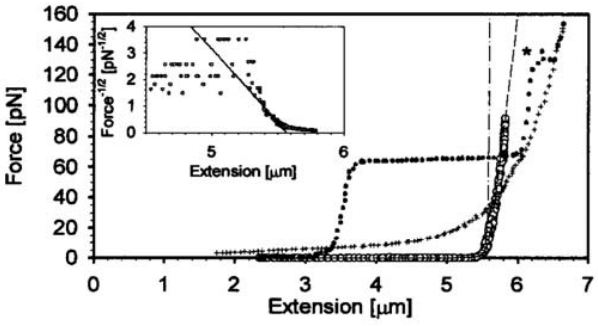
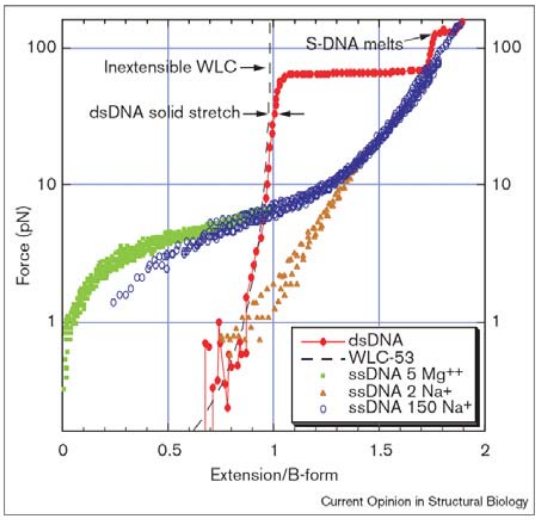


FIG. 3. Force vs. extension plot for a ssDNA–RecA filament in the presence of ATP[γS] (shown in empty circles). The dotted curve shows the F vs. extension curve for bare dsDNA, and the crosshair curve shows the bare ssDNA. To convert the dsDNA to ssDNA the dsDNA fragment was pulled to forces higher than 140 pN. At this force the second strand was released into the surrounding buffer. The dash-dotted curve displays the inextensible WLC model for a molecule with contour length 5.57 μm and a persistence length of 923 nm, whereas the dashed curve shows the extensible WLC model including a stretch modulus of 2210 pN. (*Inset*) $F^{-1/2}$ vs. extension plot for the ssDNA–RecA filament in the presence of ATP[γS]. At forces close to 5 pN, the end-to-end distance of a WLC approaches its contour length as $F^{-1/2}$, therefore, extrapolation to infinite force in an $F^{-1/2}$ vs. extension plot yields the contour length at the abscissa intercept, while extrapolation to zero extension gives an ordinate intercept equal to $(4A/k_BT)^{-1/2}$. The line indicates the mean value of the persistence length of all the ssDNA–RecA–ATP filaments measured.

Single-molecule studies of DNA mechanics
Carlos Bustamante*†§, Steven B Smith*, Jan Liphardt‡ and Doug Smith† **Current Opinion in Structural Biology** 2000, **10**:279–285

Figure 2



Force/extension behavior of dsDNA and ssDNA. Different DNA molecules were pulled with force-measuring laser tweezers [6]. Both pulling and relaxing curves are shown, so all force curves were reversible. Dashed line data (WLC-53) are from Equation 1, assuming P = 53 nm. The dsDNA curve was taken using a 10.4 kbp restriction fragment in 50 mM Na^+ and 5 mM Mg^{++} buffer [25•]. The same fragment and buffer were used to make ssDNA (labeled ssDNA 5 Mg^{++}) [40••]. The ssDNA curves in 150 mM Na^+ and 2 mM Na^+ were taken using 48 kbp λ phage DNA [6]. See text for further details.

Gaub AFM Data

Hauke Clausen-Schaumann,* Matthias Rief,† Carolin Tolksdorf,* and Hermann E. Gaub* Biophysical Journal Volume 78 April 2000 1997–2007

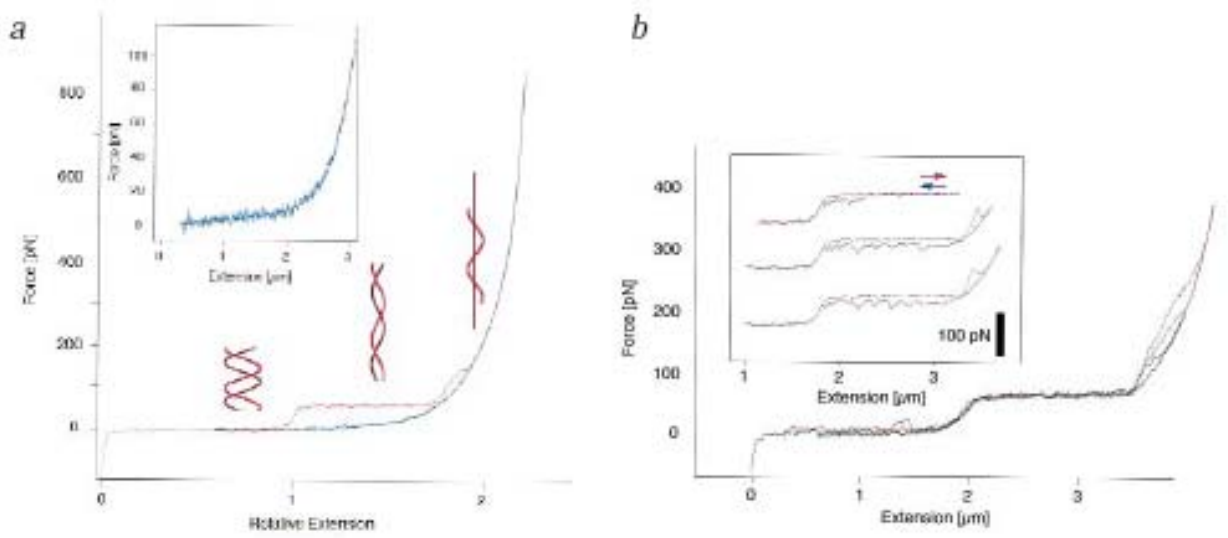


Fig. 1 Force-induced melting transition in λ-DNA. *a*, Stretching (red) and relaxation (blue) curve of a 1.6-µm-long segment of a λ-BstE II digested DNA molecule. The superimposed black curve is a force curve taken on ssDNA. Because the ssDNA strand has a different contour length (~300 nm), the curves were scaled to the same contour length before superposition. Inset: low-force range of a relaxation curve of λ-DNA previously split in the melting transition (blue curve). The black curve is a fit according to the freely jointed chain model with an additional segment elasticity using the same parameters that have been determined for ssDNA[12] (Kuhn length 8 Å, molecular spring constant per unit length 800 pN). *b*, Superposition of four extension traces of the same molecule taken at different pulling speeds (red, 3 µm s^{-1}; green, 1.5 µm s^{-1}; blue, 0.7 µm s^{-1}; black, 0.15 µm s^{-1}). Inset: a sequence of three stretching (red) and relaxation (blue) cycles performed each time on the same molecule (pulling speed: 0.25 µm s^{-1}). Partial melting of the double helix occurs during the B-S transition (first trace) and is complete after the melting transition (second and third trace). Reannealing of the two single strands leads to the characteristic shape of the relaxation traces.

stc: 39

Data From Williams/Bloomfield

Biophysical Journal Volume 80 April 2001 1932–1939
Entropy and Heat Capacity of DNA Melting from Temperature Dependence of Single Molecule Stretching
Mark C. Williams, Jay R. Wenner, Ioulia Rouzina, and Victor A. Bloomfield

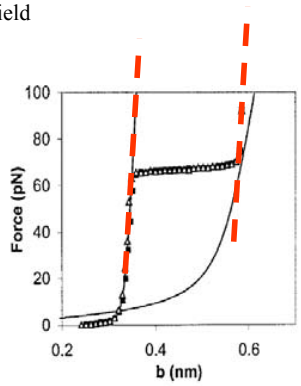
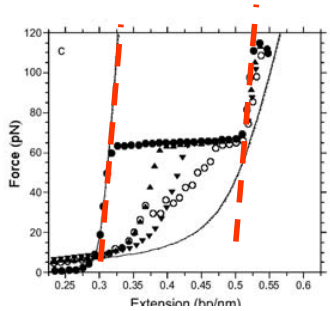


FIGURE 2 Typical room temperature force-extension (per base pair) curve for a single dsDNA molecule in 500 mM ionic strength buffer at pH 7.5. The data obtained while stretching the DNA (△) and the data obtained when the DNA is relaxed (■) are almost identical, so there is very little hysteresis under these conditions. The solid line on the left is the theoretical curve for an extensible worm-like chain (dsDNA) with a persistence length of 50 nm, a contour length of 0.34 nm/bp, and an elastic stretch modulus of 1300 pN. The solid line on the right is the curve for an extensible freely jointed chain (ssDNA) with a persistence length of 0.75 nm, a contour length of 0.56 nm/bp, and an elastic stretch modulus of 800 pN (Smith et al., 1996). The data are interpreted as a transition between dsDNA and ssDNA.

Biophysical Journal 80(4) 1932–1939

Wenner, J. R., M. C. Williams, I. Rouzina, and V. A. Bloomfield. Salt dependence of the elasticity and overstretching transition ofAcad. Sci. USA. 96:10109–10114.single DNA molecules. Biophys. J. 82:3160–3169.



during the relaxation curve (○) and subsequent stretch (▲). (*C*) Multiple stretches to high extensions produce partially ssDNA. A dsDNA molecule that has been tethered in 250 mM buffer and stretched to near the maximum force achievable with this instrument (●) displays large hysteresis and partial ssDNA character during relaxation (○). Second (▲) and third (▼) stretches increase ssDNA character, but additional stretching to high force failed to produce fully ssDNA.

Dual Binding Modes for an HMG Domain from Human HMGB2 on DNA
Micah McCauley,* Philip R. Hardwidge,y L. James Maher III,y and Mark C. Williams*zBiophysical Journal Volume 89 July 2005 353–364

Both red lines in each figure have the same slope.
They are added to show that the slopes above and below the transitions are the same

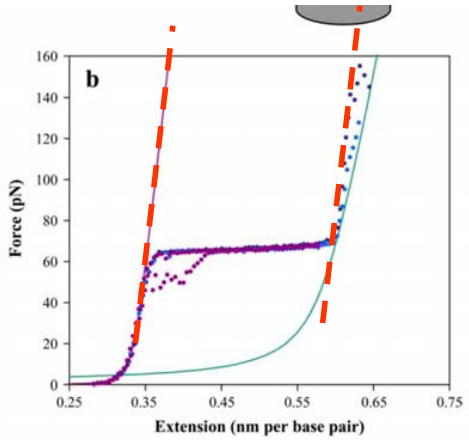
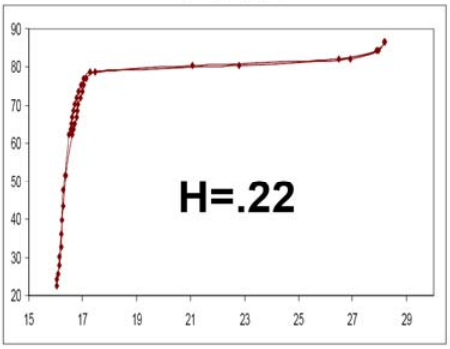


FIGURE 2 (*a*) In this optical tweezers diagram, the vertical counter propagating beams are focused on the right. Within the gray flow cell, a streptavidin-coated polystyrene bead (*open circle*) is held in the trap formed by the beams, while another is attached to the glass micropipette tip on the left. Labeled DNA molecules are stretched by moving the cell and tip relative to the trap. (*b*) Typical force extension curves for double-stranded DNA are shown as dotted lines. As the stretching force is increased, dsDNA reveals an entropic elastic response, followed by the overstretching region. The data in purple shows typical data for a full cycle of extension and relaxation, including some hysteresis upon reannealing. The data in blue and cyan show the response of the resulting single strands to yet higher forces, as the strands finally separate near 150 pN (thus there are no relaxation curves). The solid lines are DNA models for ssDNA and dsDNA, as described in the text.

Hysteresis Differs for Monovalent Cations

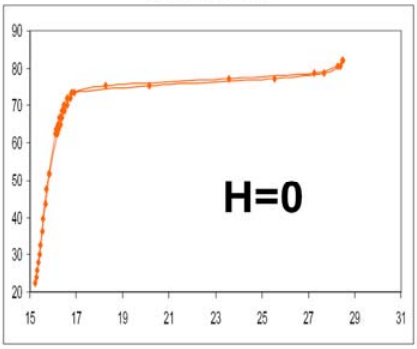
All pulling is 3'3'

LiCl



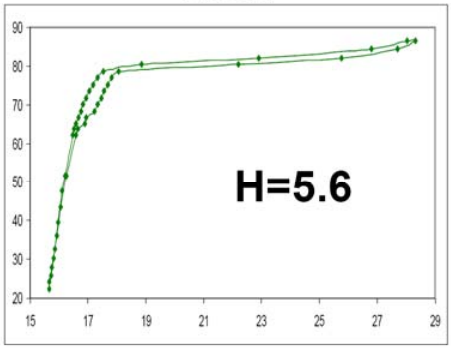
Ave H=0.11+-0.16

NaCl



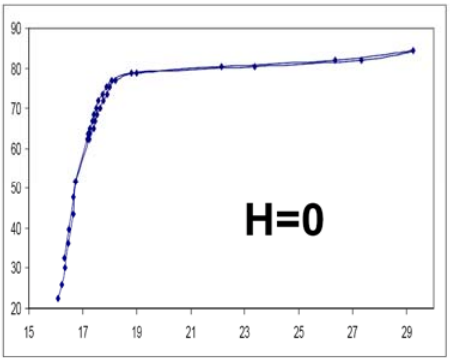
Ave H=0.23+- 0.31

KCl



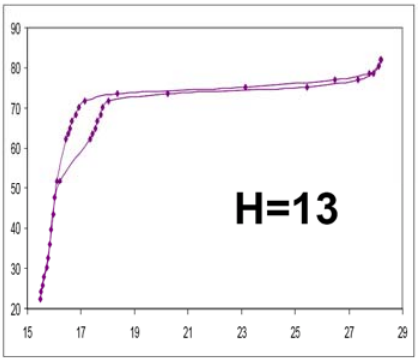
Ave H=7.3+- 5.0

RbCl



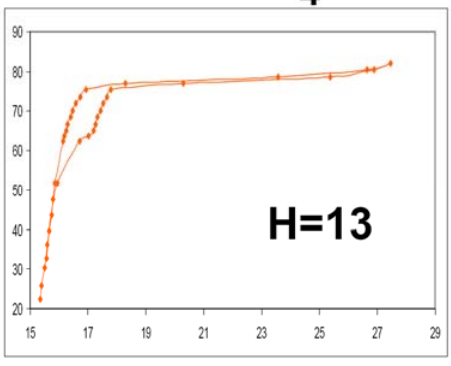
Ave H =0.07+- 0.14

CsCl



Ave H=9.3+- 11

$NaClO_4$



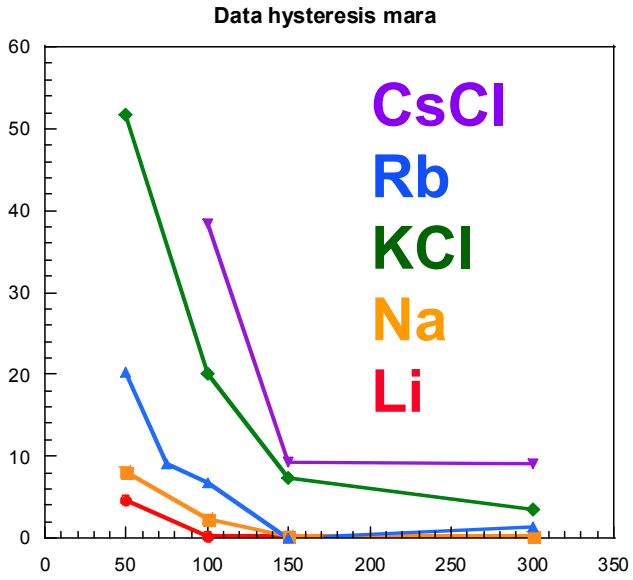
Ave H=8.6+- 6.3

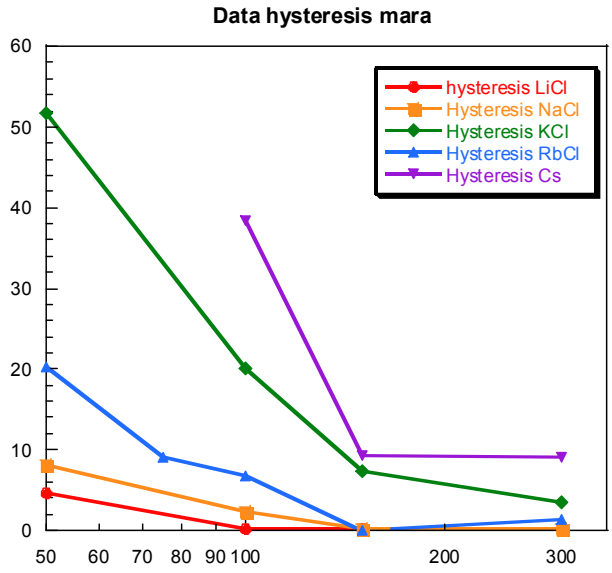
The anion matters too

This hysteresis is not due to nicks, though as theory predicts we find that adding nicks can create hysteresis for 3'3' pulling in 150 mM NaCl where there is no hysteresis in the absence of nicks
Note: 1 D finite system at finite temperature with Hamiltonian heterogeneity due to sequence

Hysteresis vs Concentration

(Hysteresis is a measure of screening)





Color Corresponds to Atomic Weight Screening Decreases with Weigth Except RbCl screens better than KCl

Table 1. Summary of the Experimental Results for the Different Cations in 150 mM Salt and Tris 10 mM pH 7.5

salt	F_u (pN)	F_z (pN)	T_{melt} (°C)	H (pN μm)	F_o (pN)
LiCl	19.1 ± 1.9	13.6 ± 1.6	95.3 ± 2	0.11 ± 0.16	83 ± 2
NaCl	16.6 ± 2.0	11.9 ± 1.3	93.4 ± 2	0.23 ± 0.31	82 ± 4.3
KCl	18.4 ± 3.7	13.6 ± 2.7	93.2 ± 2	7.3 ± 5.0	79 ± 4.9
RbCl	17.8 ± 0.8	12.5 ± 0.5	93.2 ± 2	0.07 ± 0.14	83 ± 3.9
CsCl	17.5 ± 2.9	12.7 ± 2.7	92.6 ± 2	15.4 ± 12.3	79 ± 4.3
$NaClO_4$	17.2 ± 3.1	12.7 ± 2.5	93.5 ± 2	8.6 ± 6.3	79 ± 3.3

New Conformations of dsDNA Depend on Ends to which Force is Applied

Genetica **106:** 75–84, 1999.
Modelling DNA stretching for physics and biology

Richard Lavery & Anne Lebrun

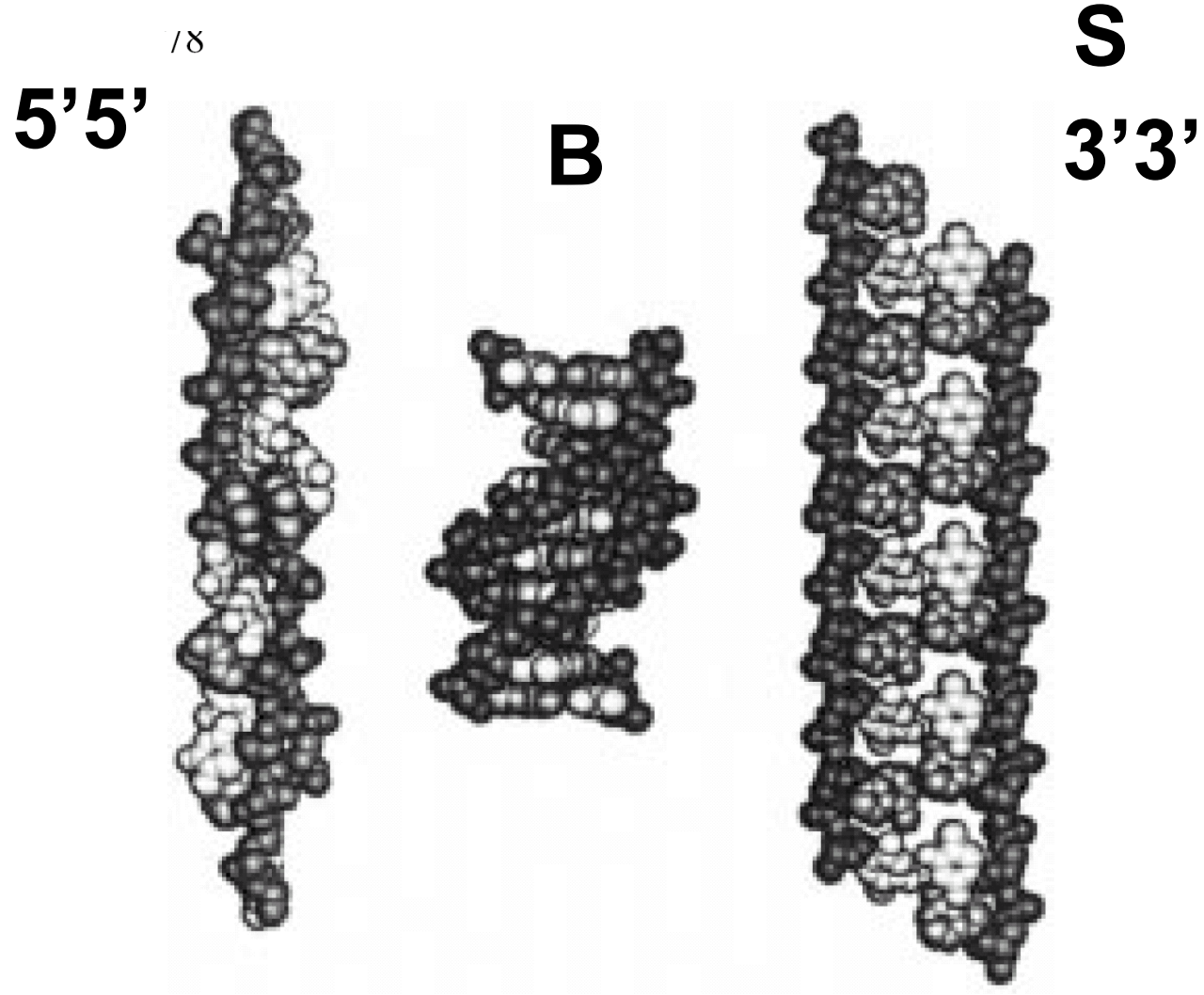
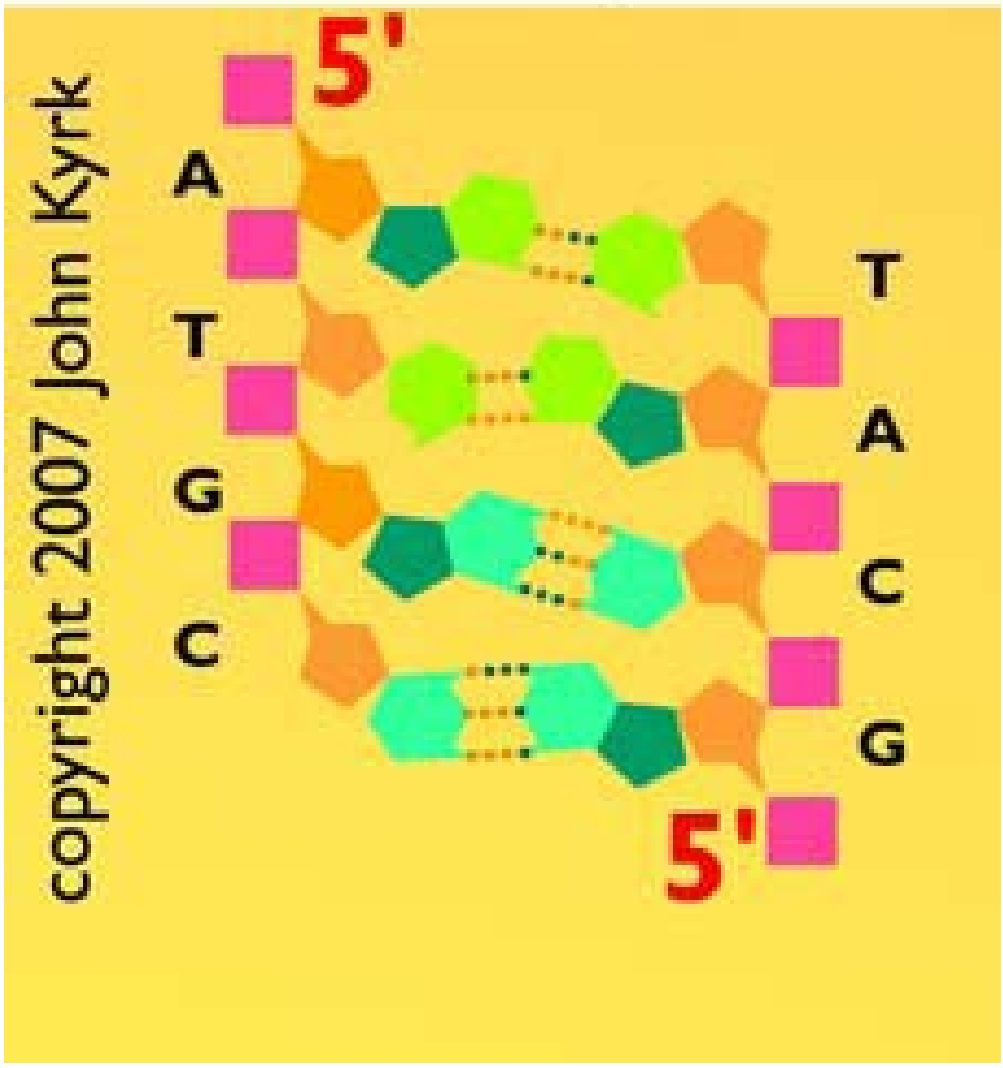


Figure 2. Space-filling models of stretched DNA (left: 5′ ends constrained, right: 3′ ends constrained) compared with a canonical B-DNA conformation having the same number of base pairs (centre).

stc: 43

Why does the pulling technique matter?

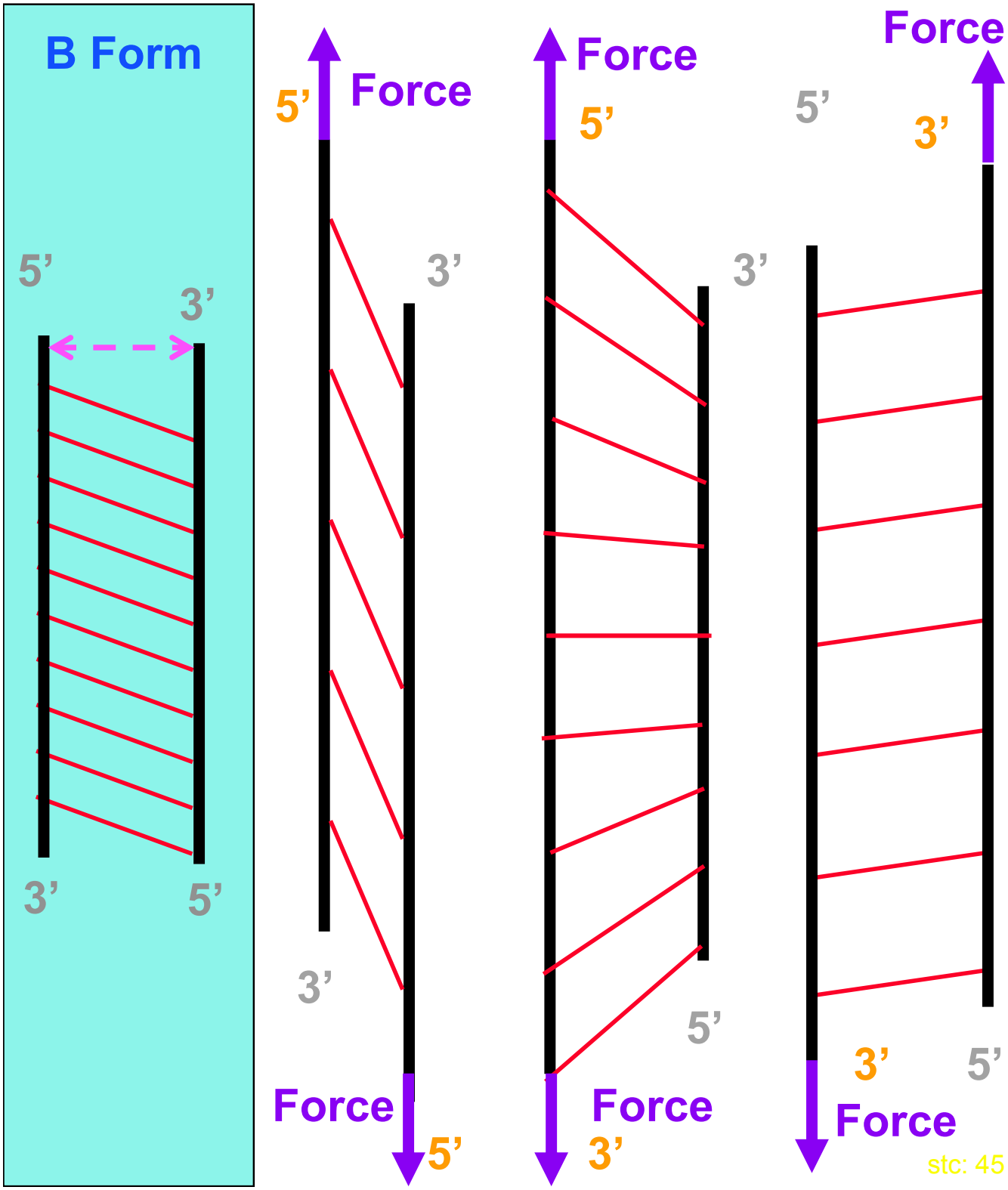
Base Pair Tilt



Basepair tilt breaks the symmetry just as helical twist breaks the rotational symmetry giving underwinding and overwinding meaning

http://www.johnkyrk.com/DNAreplication.html

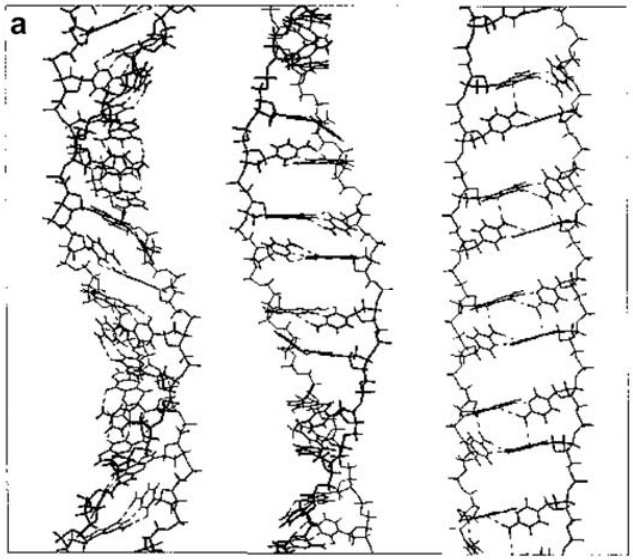
Overstretched Forms



3’3’ and 5’5’ Overstretch for CG

2260–2267 *Nucleic Acids Research, 1996, Vol. 24, No. 12*
Modeling extreme stretching of DNA Anne Lebrun and Richard Lavery*

3’3’



5’5’

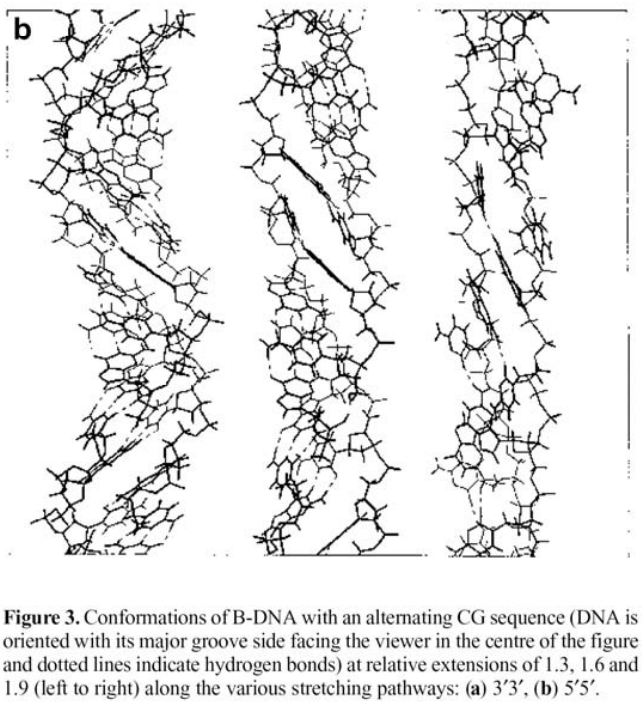


Figure 3. Conformations of B-DNA with an alternating CG sequence (DNA is oriented with its major groove side facing the viewer in the centre of the figure and dotted lines indicate hydrogen bonds) at relative extensions of 1.3, 1.6 and 1.9 (left to right) along the various stretching pathways: **(a)** 3′3′, **(b)** 5′5′.

stc: 46

Constant Interphosphate Distance

(both overstretched forms have the same length ~ 1.7 B form)

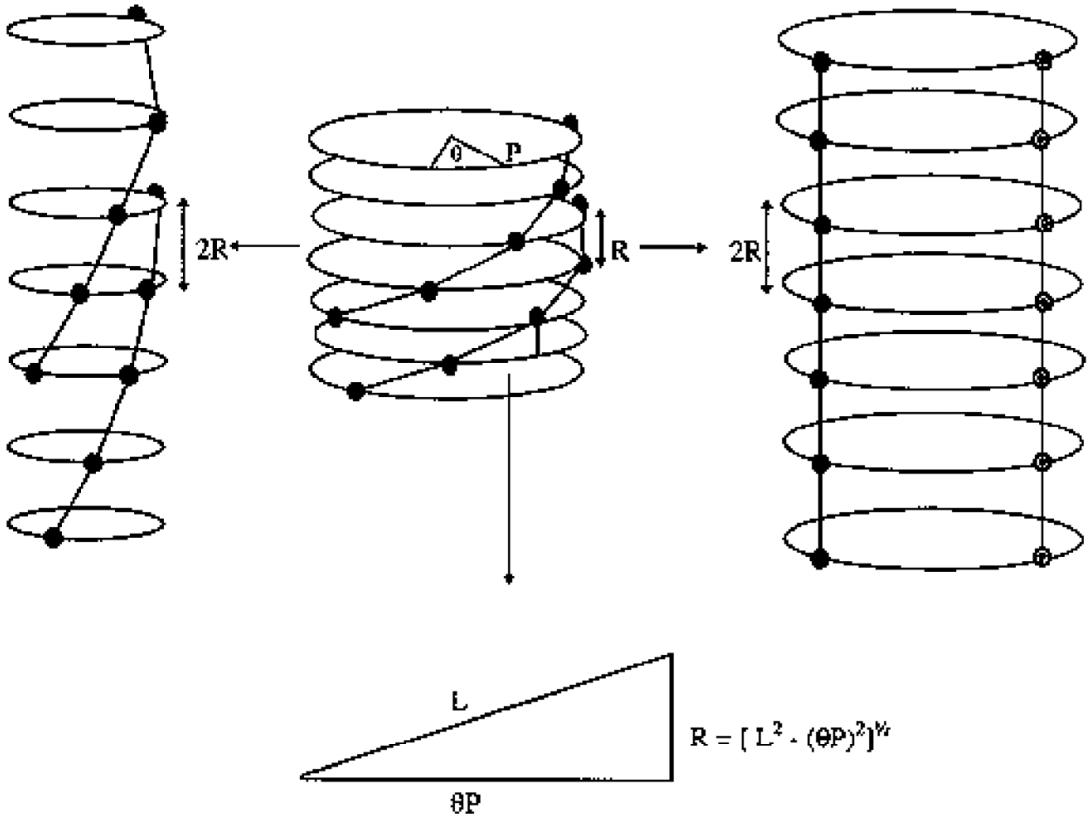


Figure 6. Schematic model of DNA stretching: maintaining a constant inter-phosphate distance L within each strand of the duplex, stretching to twice the normal rise R can be achieved by reducing the twist angle θ or by reducing the radius P of the duplex.

2260–2267 *Nucleic Acids Research, 1996, Vol. 24, No. 12*

Modelling extreme stretching of DNA Anne Lebrun and Richard Lavery*

stc: 47

Predicted Structures from the Olson Group

J. Mol. Biol. (1999) **289**, 1301–1326

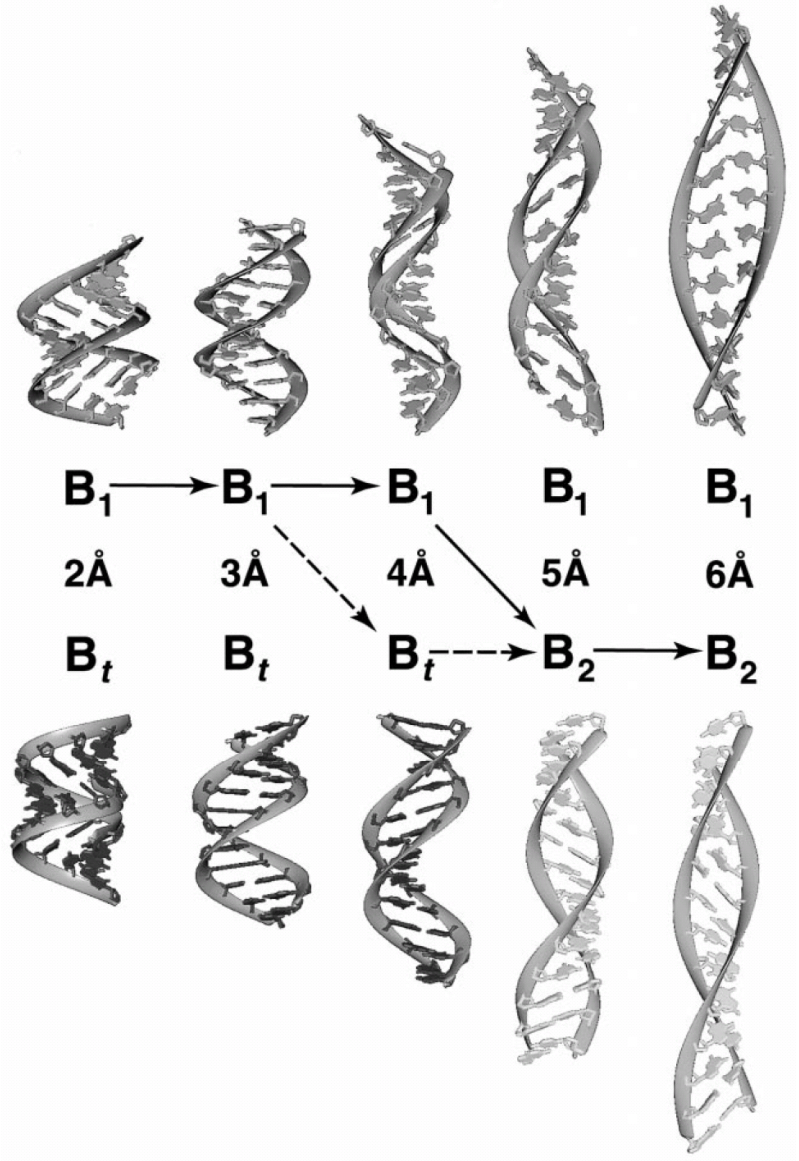


Figure 7. Ribbon representations of simulated *B*-DNA stretching. See the legend to Figure 6.

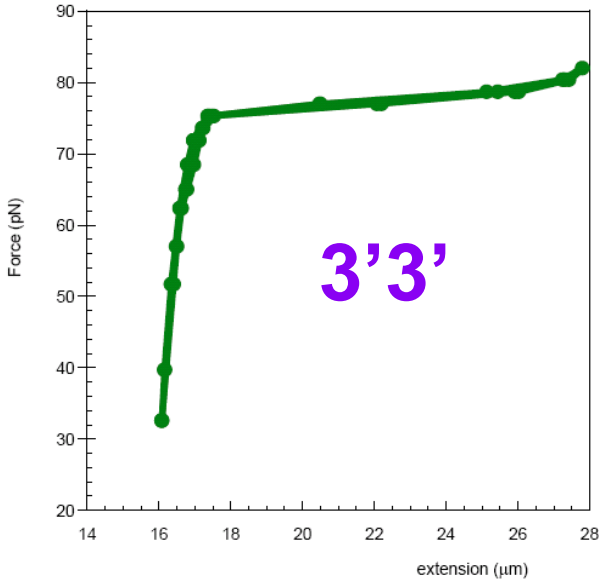
Figure 7 captions refers to figure 6 caption with A replaced by B

Figure 6. Ribbon representations, generated with `MidasPlus` (Ferrin *et al.*, 1988), of the simulated stretching of *A*-DNA structures. Side views of the lowest energy structures at the specified values of helical rise are shown. The arrows designate the putative conformational transitions suggested by the energy profiles in Figure 1. Chains are oriented so that the minor groove is always shown in the upper part of each duplex. Sugar-phosphate backbones of duplex structures are represented by gray ribbons, and base-pairs are color-coded according to conformational family: cyan, canonical A_1 and B_1 structures; magenta, A_3 and B_2 forms; ochre, A_k duplex; green, B_t conformations.

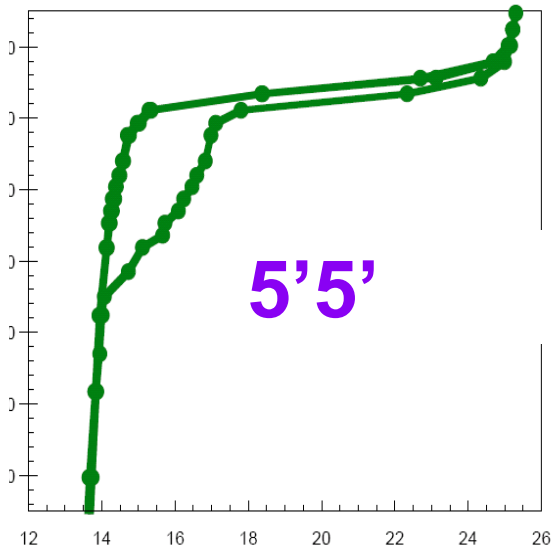
Overstretching Force and Extension are the Same

Hysteresis in Overstretched Force vs Extension DNA depends on the Pulling Technique

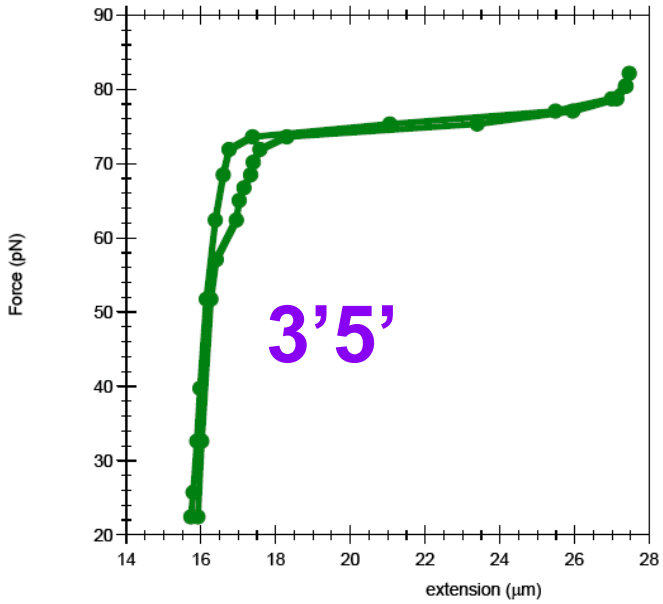
(Fc same +- 1.5% extension same +- 3%)



0.1 +- 0.3 pN μm



13.7 +- 8 pN μm



5.7 +- 4 pN μm

stc: 49

Not in Agreement with Predictions for Short Regular Sequences

– **Different forces for short regular sequence, but similar for long random sequences**

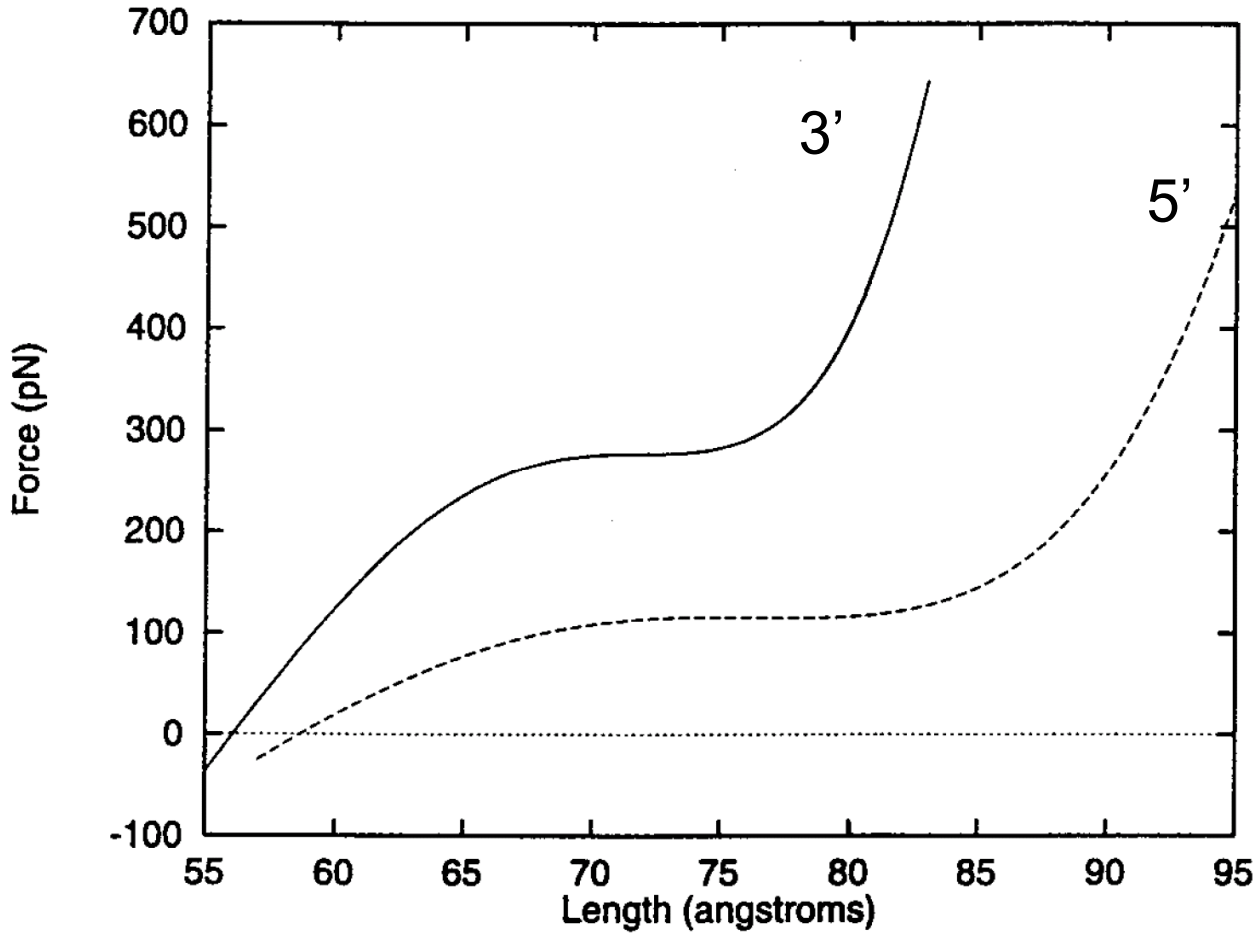
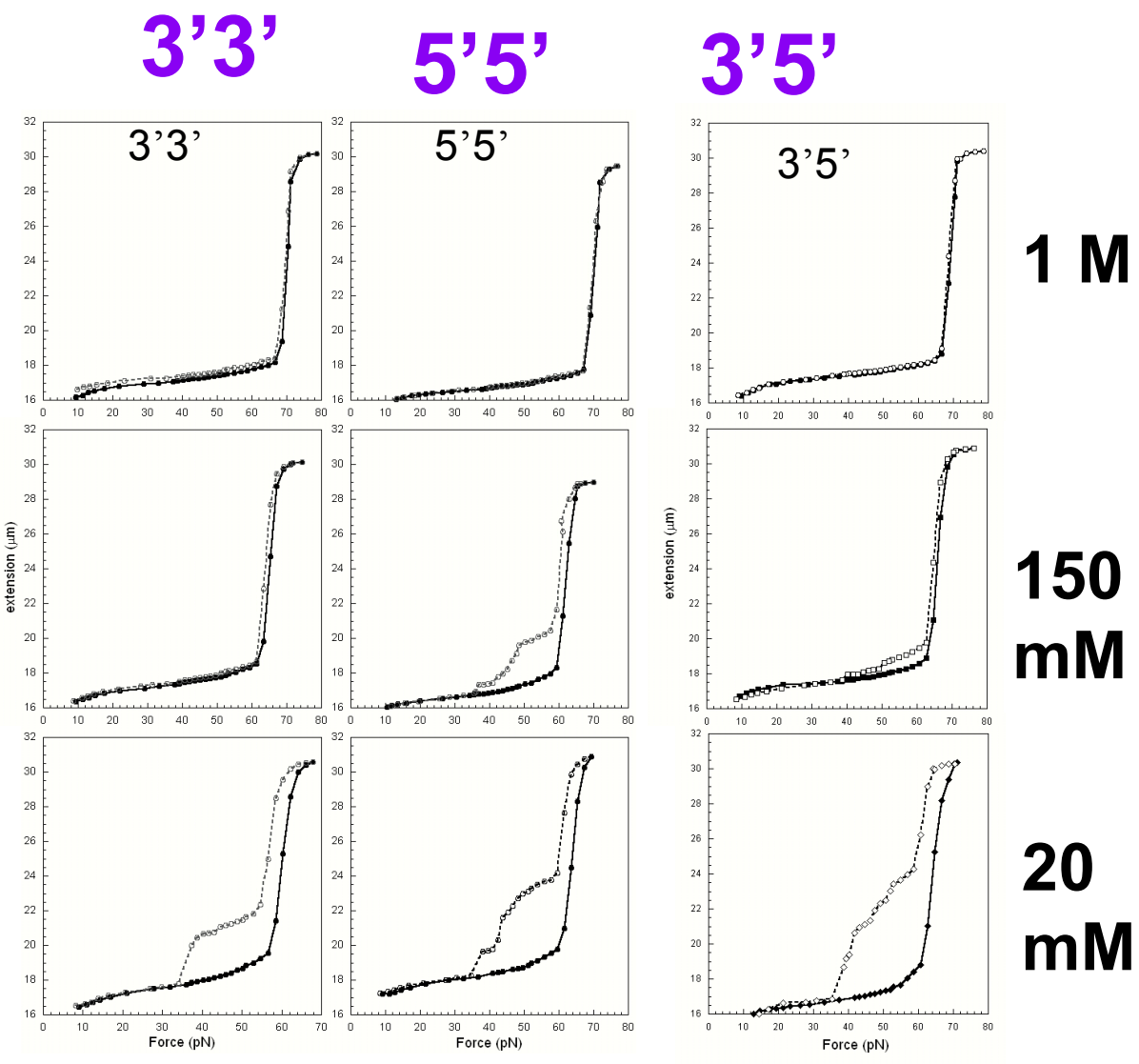


Figure 5. Force vs length curves obtained by derivation of the energy curves in figure 5 (dotted line: 5' ends constrained, solid: 3' ends constrained).

Genetica **106:** 75–84, 1999. **Modelling DNA stretching for physics and biology**
Richard Lavery & Anne Lebrun*

All Pulling Techniques are Hysteretic at Low Salt and Non-Hysteretic at High Salt

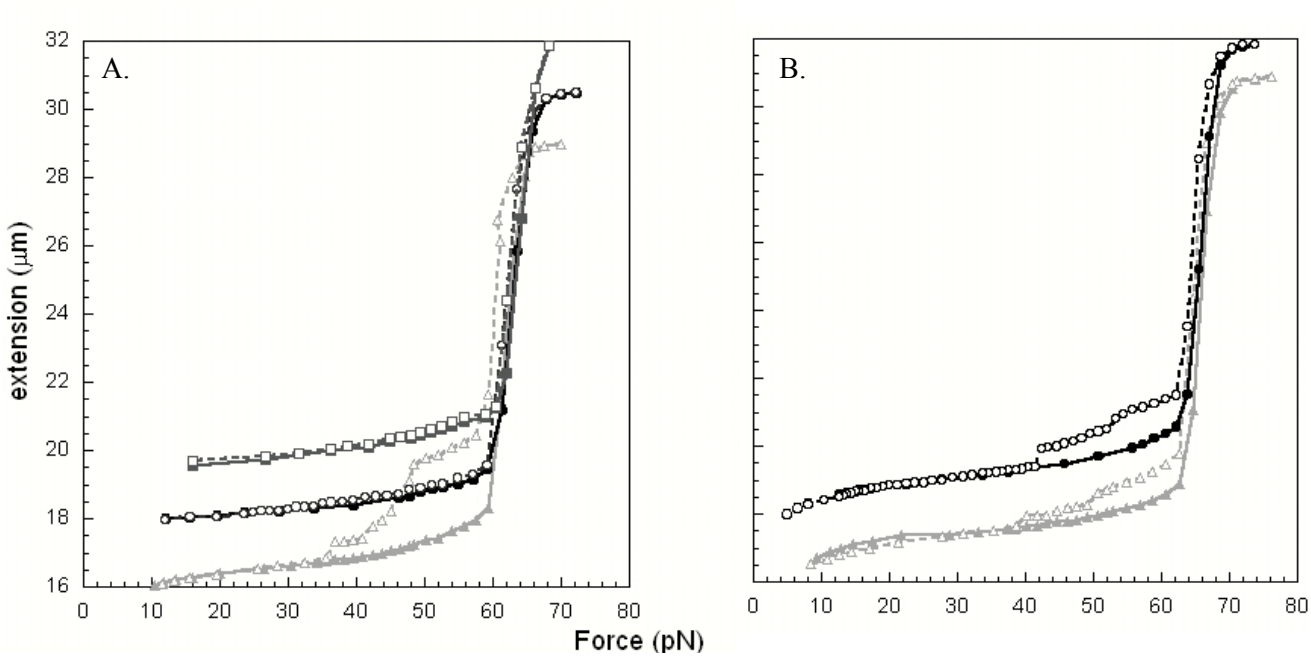


Hysteretic if screening is inadequate

If nicks are present, even 3’3’ 150 mM can become hysteretic
Overstretching has Metastable States for both increasing and decreasing Force

stc: 51

Shear Force is Distributed Over Thousands of Bases 3’5’ is not 3’ at one end and 5’ at the other

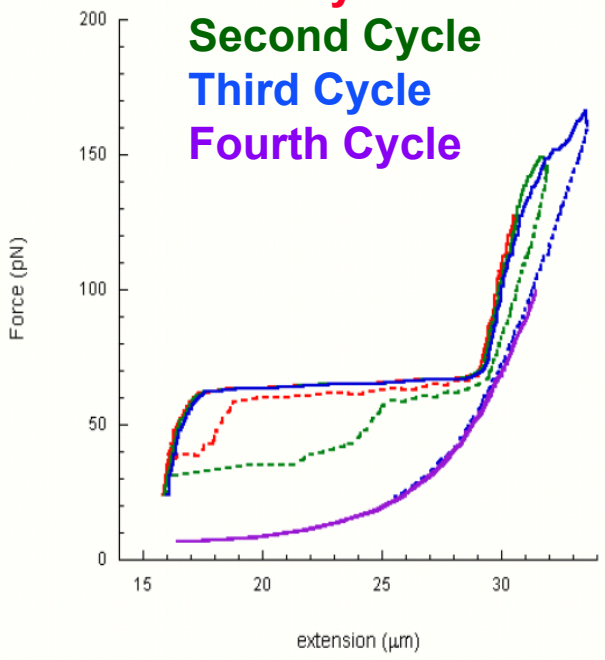


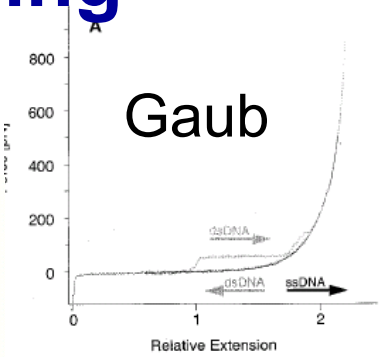
5’5’ contains rotated/open Bases that interact with Glyoxal and cyclodextrin

5’5’ does not contain rotated/open Bases that interact with Glyoxal and cyclodextrin
3’3’ does not contain rotated/open bases

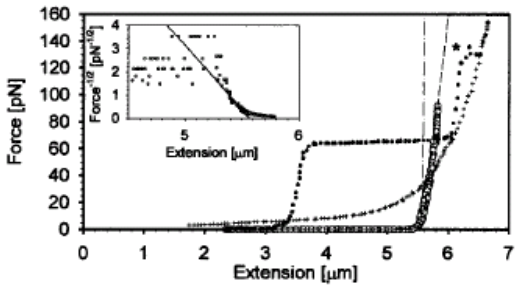
Overstretch to New dsDNA Conformation Followed by Shearing

J. Morfill, F. Kuhner, K. Blank, R. A. Lugmaier, J. Sedlmair, and H. E. Gaub, Biophys. J. **93**, 2400 (2007).



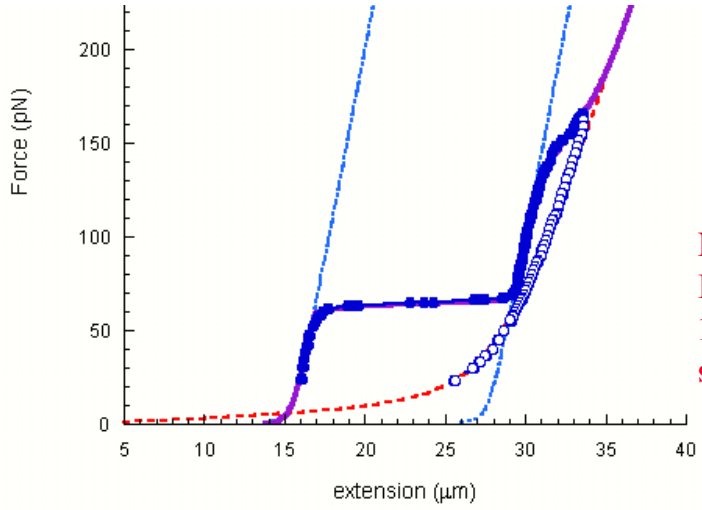


10112 Biophysics: Hegner *et al.*



Bustamante

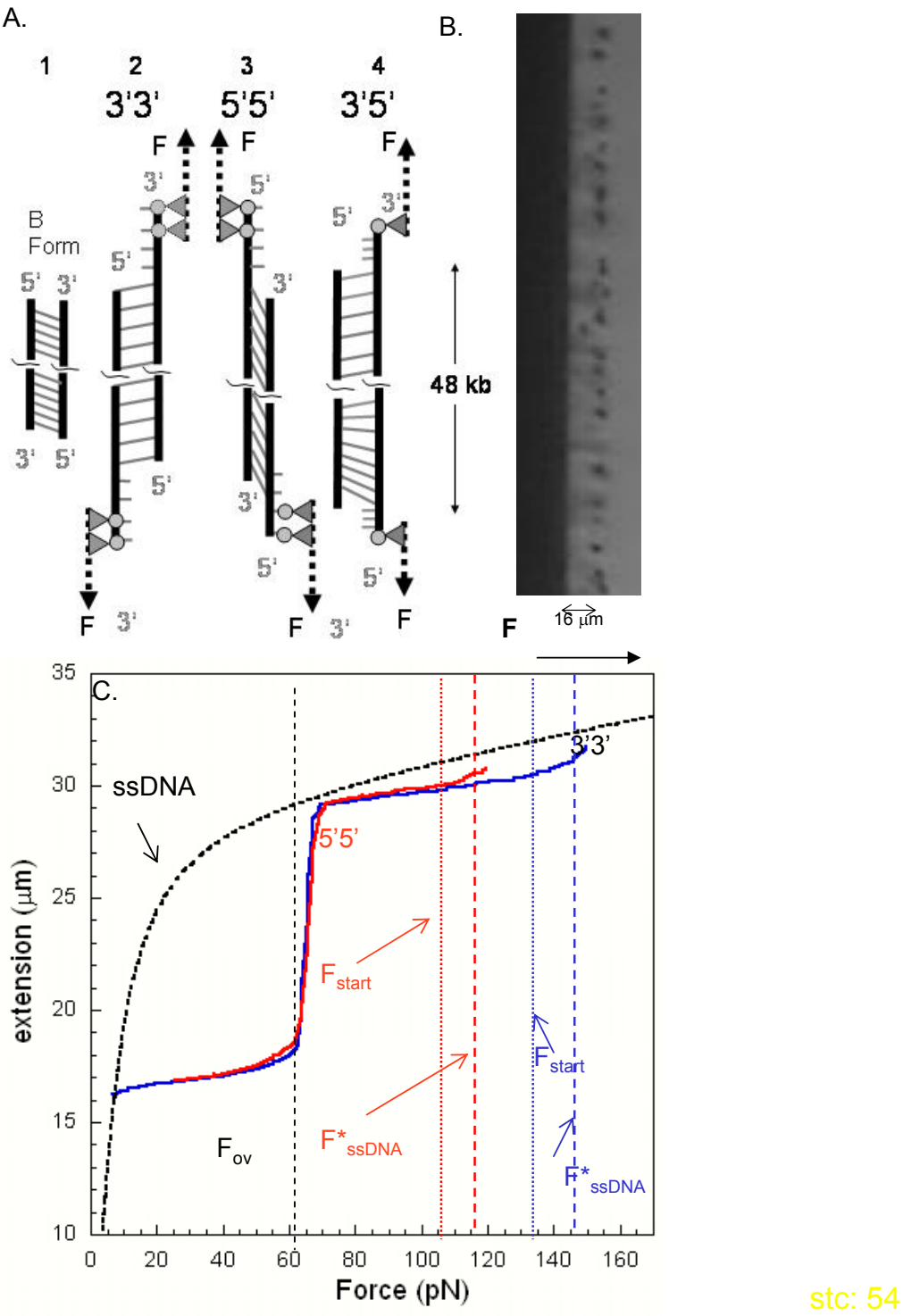
Proc. Natl. Acad. Sci. USA
Vol. 96, pp.



ssDNA theory
dsDNA theory
Note displaced theory fits above overstretch

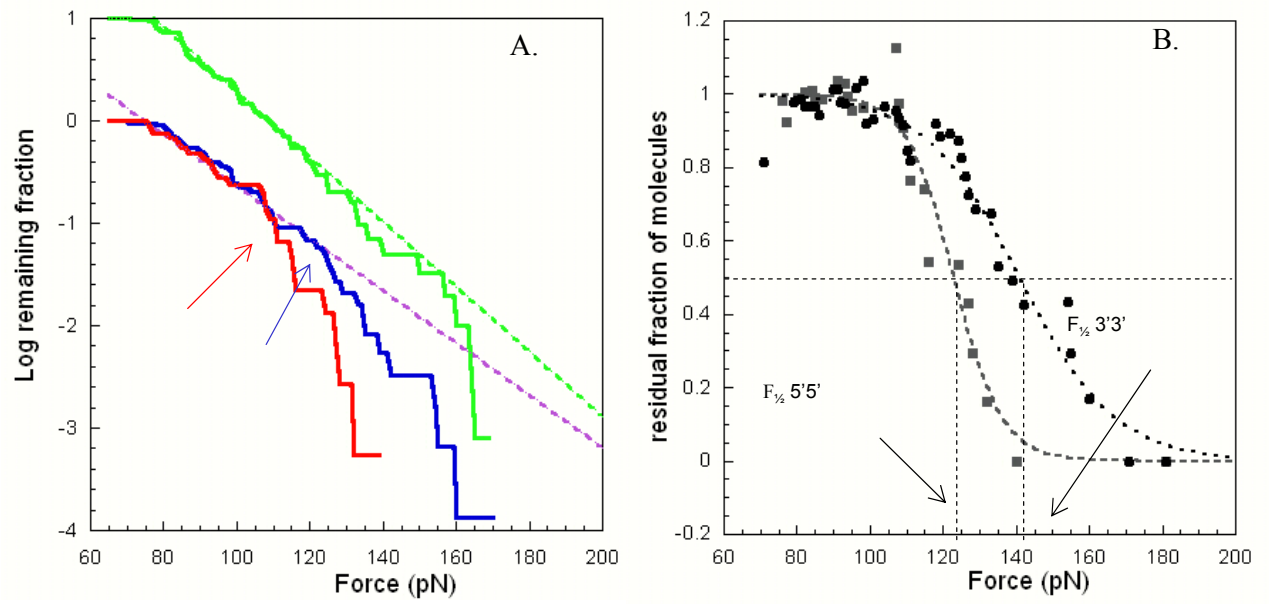
Note ssDNA <5% for First curve, but hystereis is 13%, so hysteresis not due to ssDNA

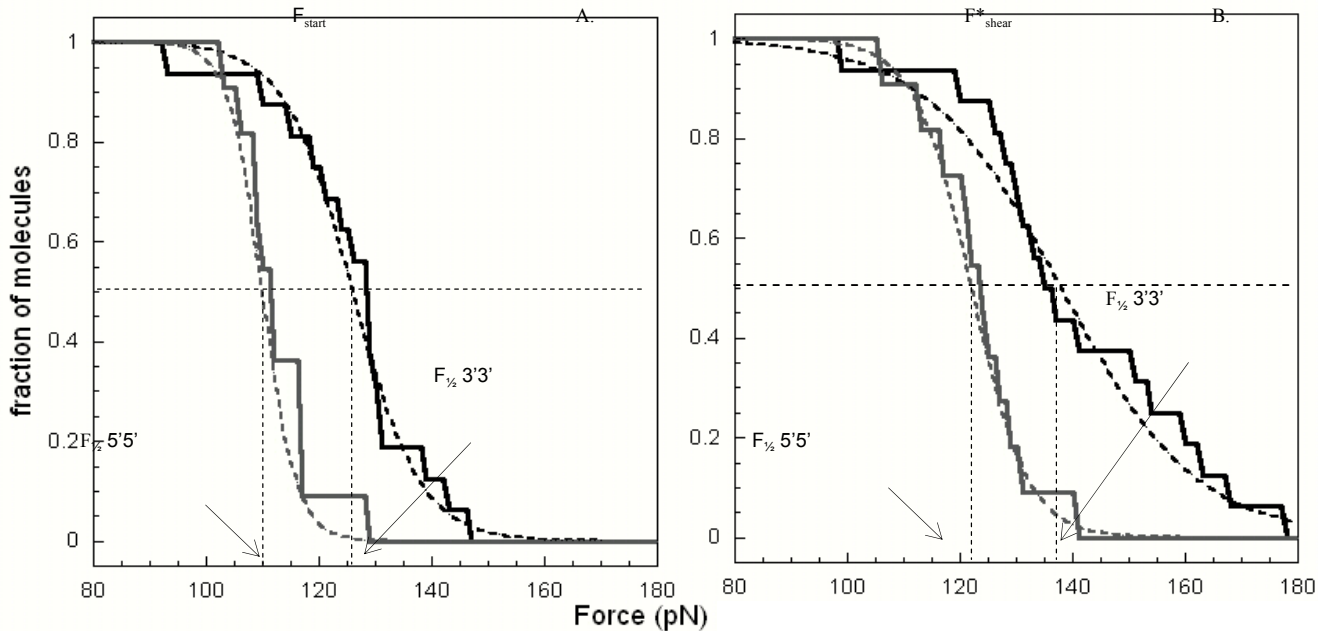
Overstretched 3’3’ and 5’5 Shear at Different Forces



stc: 54

Different Test of 3'3' and 5'5' Shear Forces



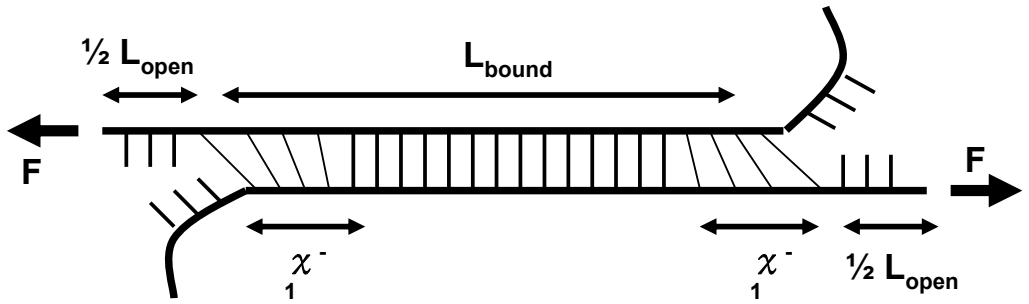


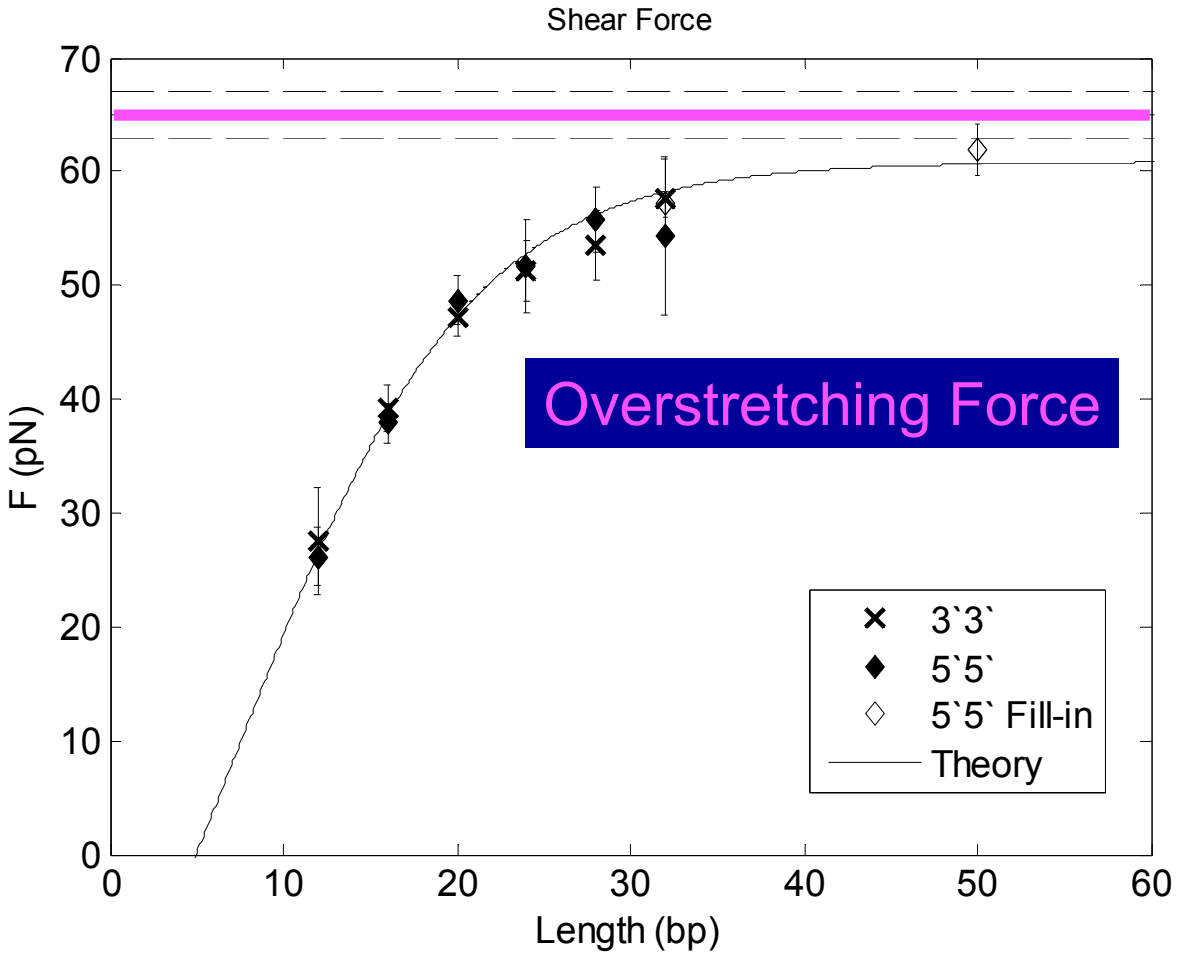
Note Gaub showed 5'5' sheared at lower force than 3'3' if the pulling rate was high, but no difference for low pulling rate

Albrecht CH, Neuert G, Lugmaier RA, Gaub HE (2008) Molecular force balance measurements reveal that double-stranded DNA unbinds under force in rate-dependent pathways. *Biophys J* 94:4766-4774.

stc: 55

Short 3'3' and 5'5' Sequences with a shear force < F_{over} Shear at the same Force

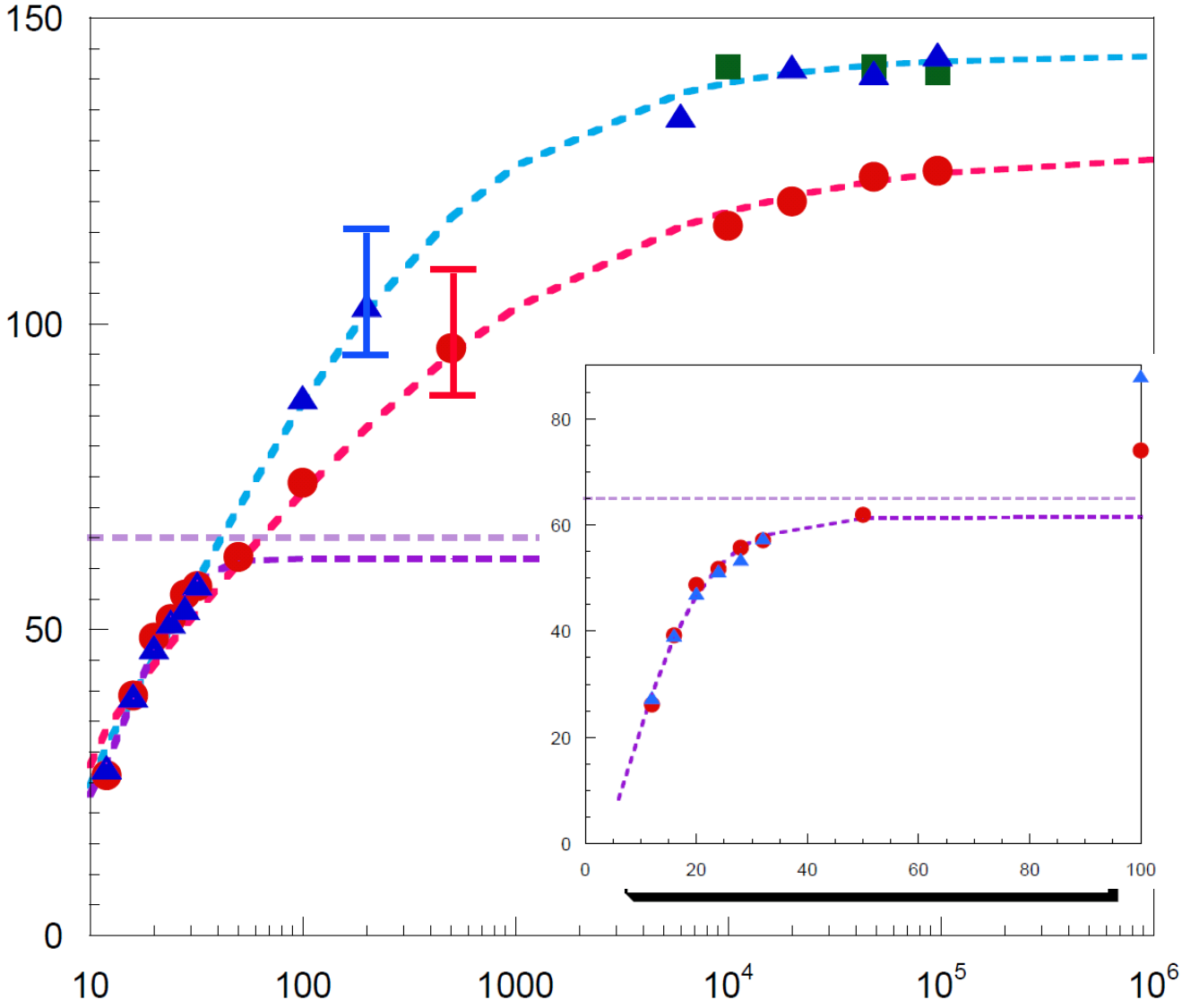




f1 = 3.9 pN, *Lopen* = 7 bp, and *c-1* = 6.8 bp. This value of *c* corresponds to a ratio *Q/R* = 92.5.

stc: 56

Preliminary Shear Force vs Log Length
3'3', 5'5', and 3'5'



f1B:= 3.5 LopenB:= 5.6 chisq:= 0.35 deGennes Length := 7.8

Overstretched Data not well described by deGennes Model.

Lambda has force distribute over thousands of basepairs and a very low bp binding force, consistent with 3'5' not being 3' on one end and 5' on the other shown by hysteresis and shearing

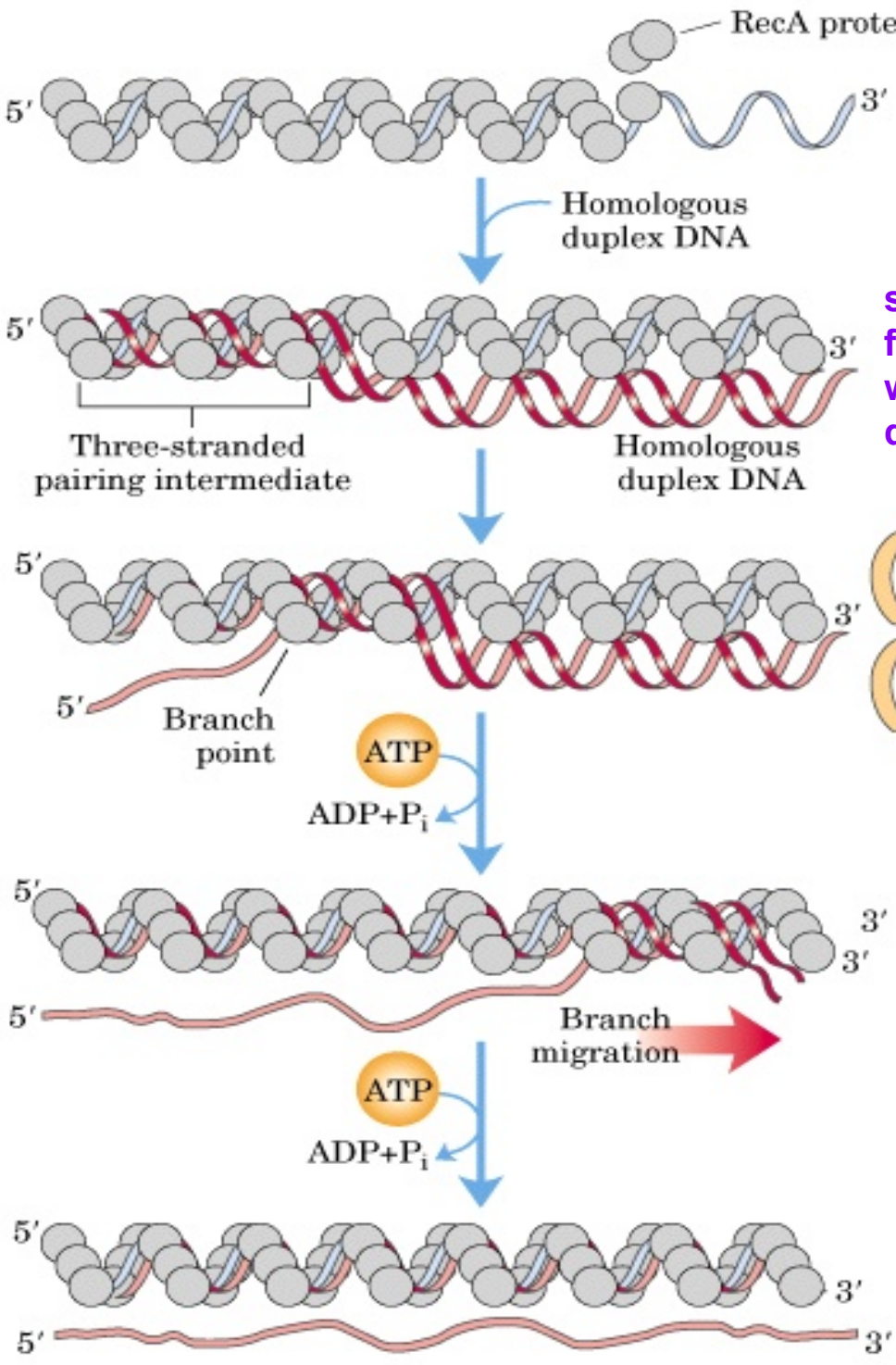
Note: "overstretched state" properties continue to change with applied force

stc: 57

Biologists' Questions

- How much tension is on dsDNA in vivo?
- If it is low, why do exotic high force states matter?

RecA and Strand Exchange



RecA covers ssDNA bound in site I

ssDNA/RecA filament joins with matching dsDNA in site II

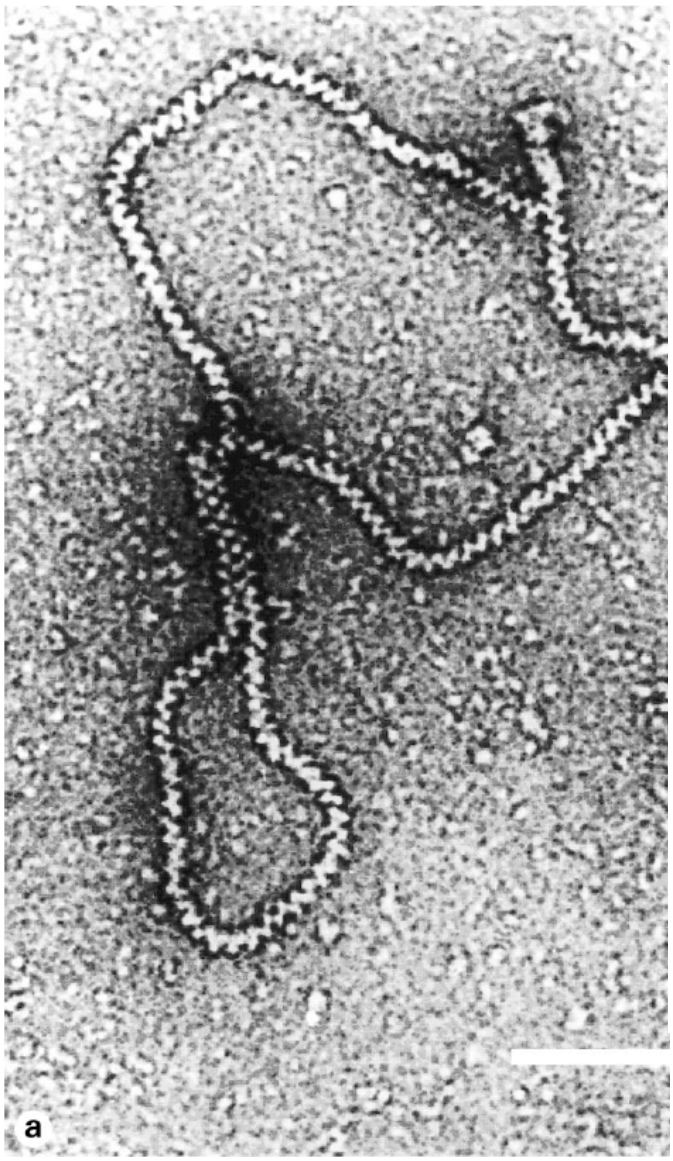
one strand from site II joins ssDNA in site I

dsDNA in site I, ssDNA in site II with ssDNA from site II released at then end, possibly by ssB

Kowalczykowski SC., Nature. 2008 May 22;453(7194):463-6.

stc: 59

DNA bound in RecA is 1.5 B-form length/Basepair Binding Differs for Different Pulling Techniques



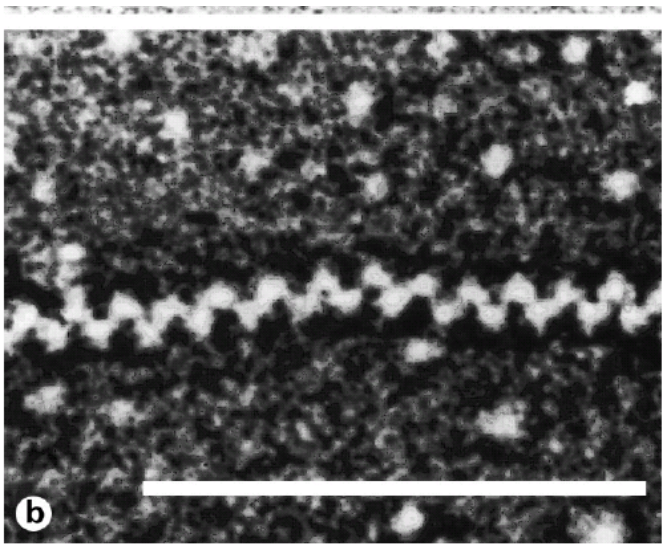
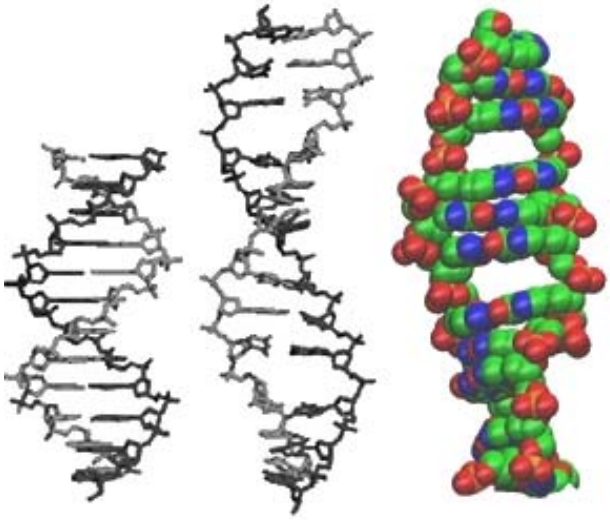


Fig. 1. Electron micrographs of a nucleoprotein filament. The circular ssDNA from bacteriophage M13 has been incubated with an excess of RecA protein (1 RecA monomer/2 bases), Mg^{2+} (5 m*M*) and ATP-γ-S (2 m*M*). **a** RecA-saturated nucleoprotein filament. **b** Detail of the helical structure of the filament. Bar corresponds to 100 nm.

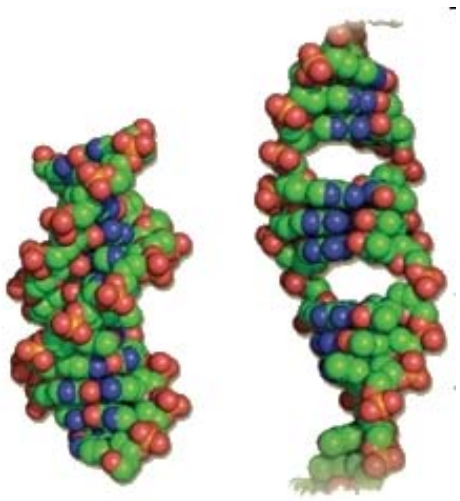
ComPlexUs 2003;1:89–99; DOI:10.1159/000070465

stc: 60

DNA/RecA Structure
Theory and Crystallography



Model of RecA-bound DNA predicted in 1998 using the Jumna software.
Bertucat, JBSD 1999, Biophys J 1999
Prévost&Takahashi, Q R Biophys 2004



Structure of RecA-bound DNA solved by crystallography in 2008
Chen et al., Nature 2008, 453, 489
(from Kowalczykowski, Nature 2008, 453, 463)

http://www-lbt.ibpc.fr/LBT/index.php?page=chantal-2&hl=fr

New Experiments:

- RecA Binding Differs for Different Pulling Techniques
- New Model of RecA Function where almost all features are explained by the mechanical properties of DNA
- RecA suggest an upper bound for DNA tension in vivo

Thanks to Theorists

We became involved with Biophysics due to a suggestion from David Nelson, and most of our work has been inspired by issues raised by theorists

The End