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

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Single-Molecule Manipulation of DNA

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Outline

I ssDNA Melting

- Background and motivation
- Single-molecule manipulation and force measurement of ssDNA using AFM
- ssDNA unstacking pathways
- Constant velocity and constant force measurements
- Mechanisms of overstretching

II dsDNA Melting

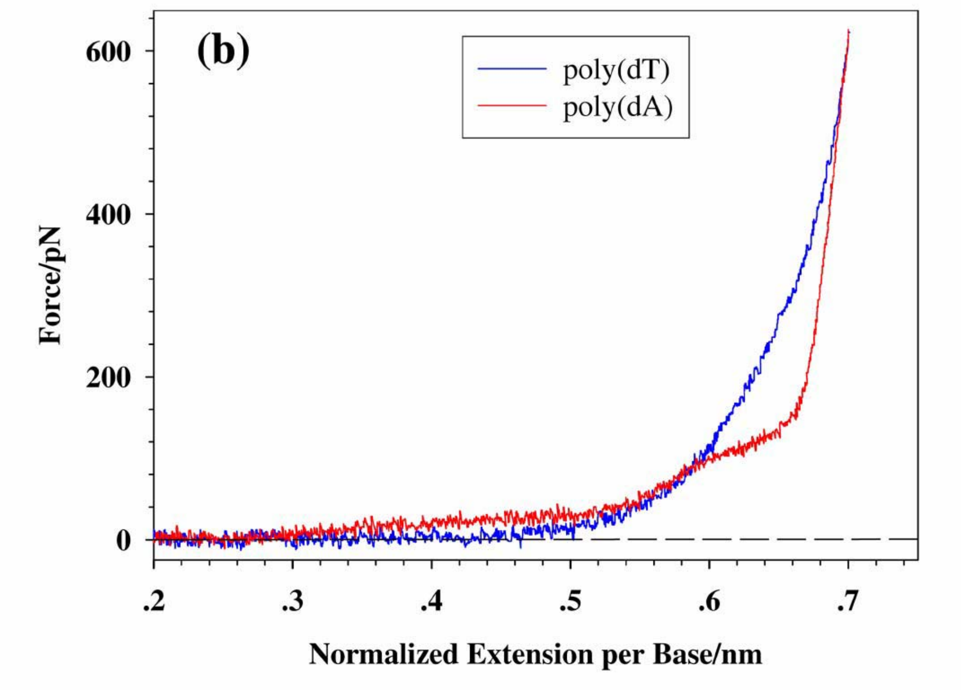
- DNA-linked gold nanoparticle phase transition
- Overstreching and mechanical melting of dsDNA

Overstretching of ssDNA

Background and Motivation

- Conformational and energetic changes of stretched DNA are relevant to biological functions
- During processes such as replication, transcription, and repair, ssDNA is stretched and stabilized by coupling with proteins to serve as an intermediate state
- Ribonucleoprotein complexes in virus (Influenza A, H1N1 virus that causes the “Swine Flu”)
- Implication in microarray analysis
- Base stacking energetics without interacting molecules or melting
- PolydA has two transitions during overstretching transitions
- Elasticity of overstretched ssDNA

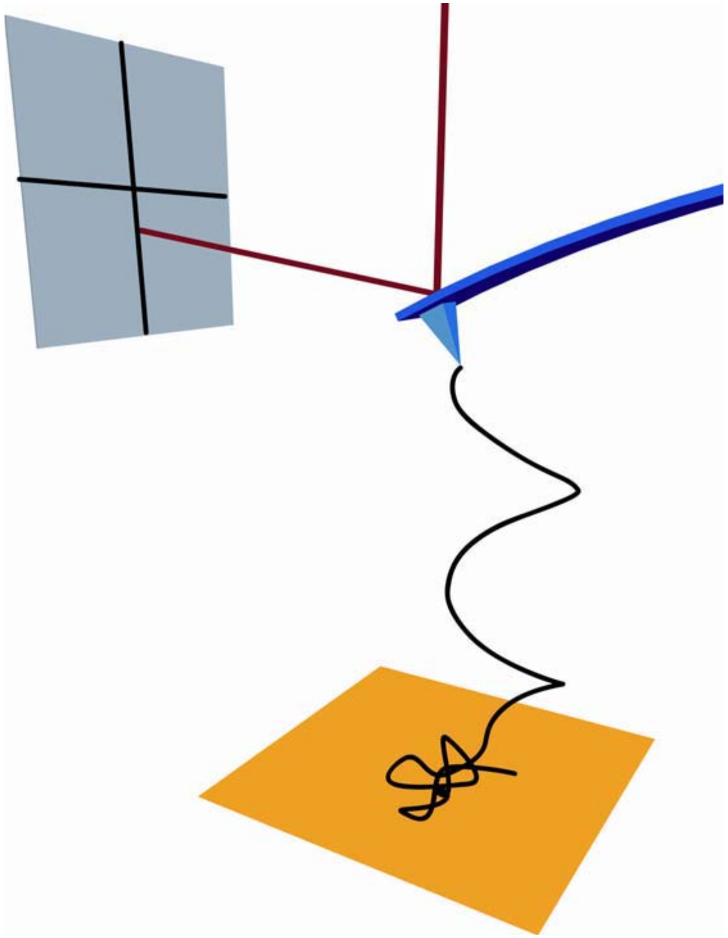
PolydA Exhibit Unique Stacking Behavior



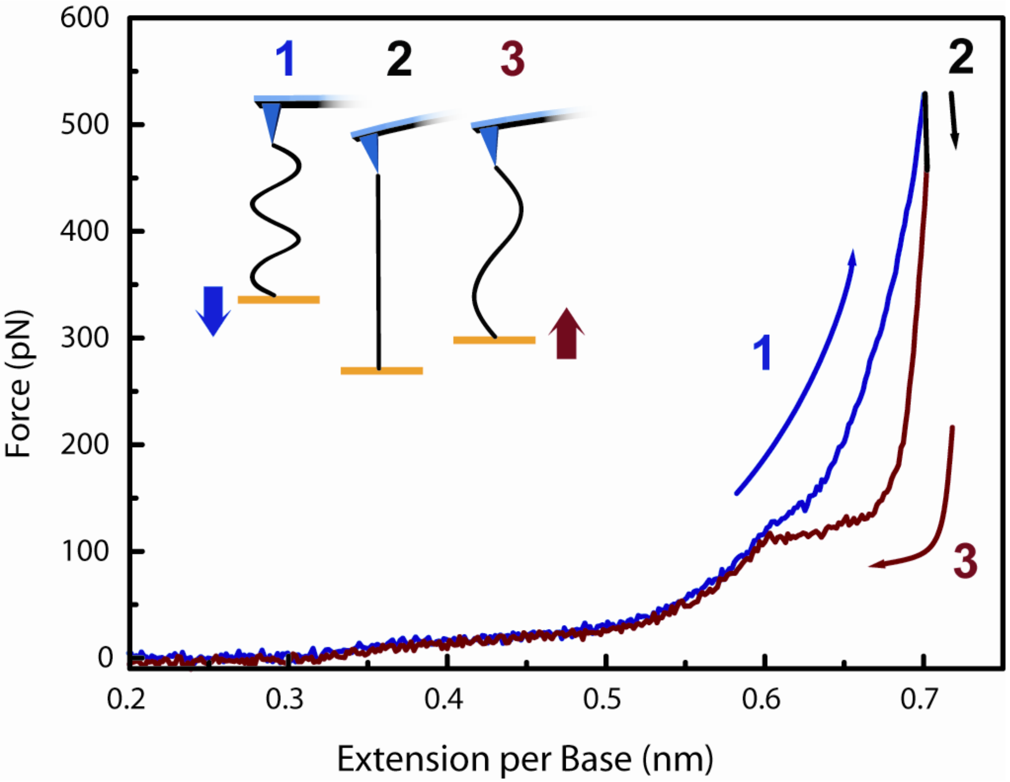
Ke *et. al.*, **Phys. Rev. Lett**., 99 (2007) 018302.

Pulling Single-Molecules Using Atomic Force Microscopy

- Nanobiology approach to probe biomolecular interactions
- Manipulation and measurements at the single-molecule level
- The end-to-end distance (z) and the force (f) on the molecule were measured

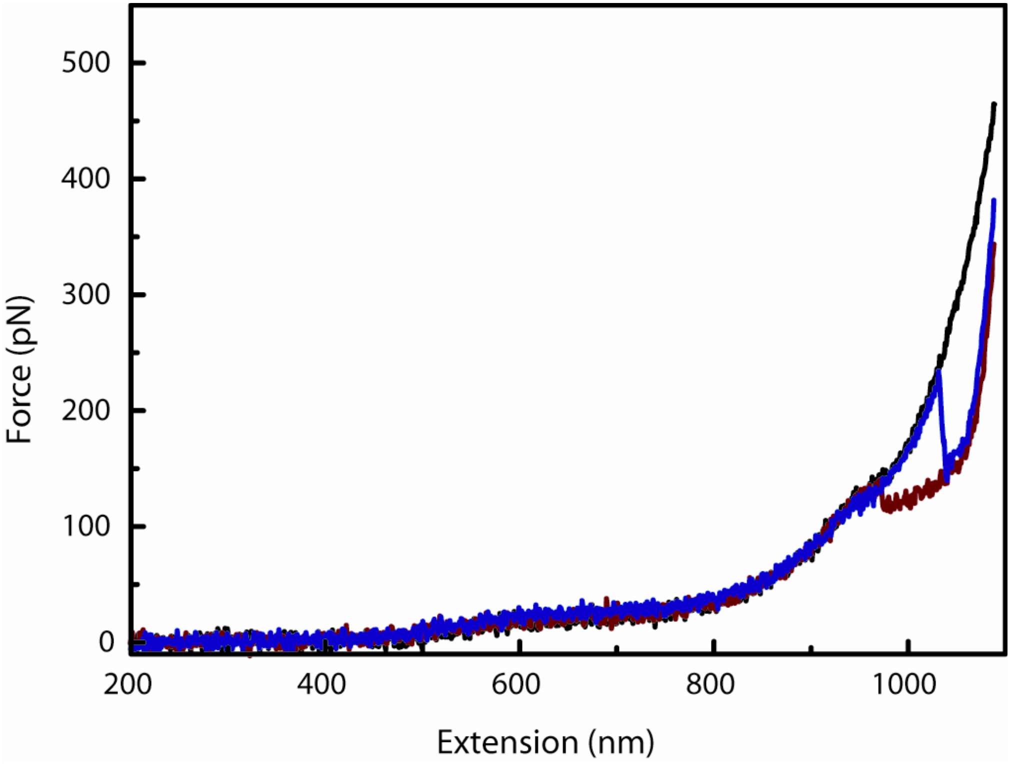


Stacking and Unstacking



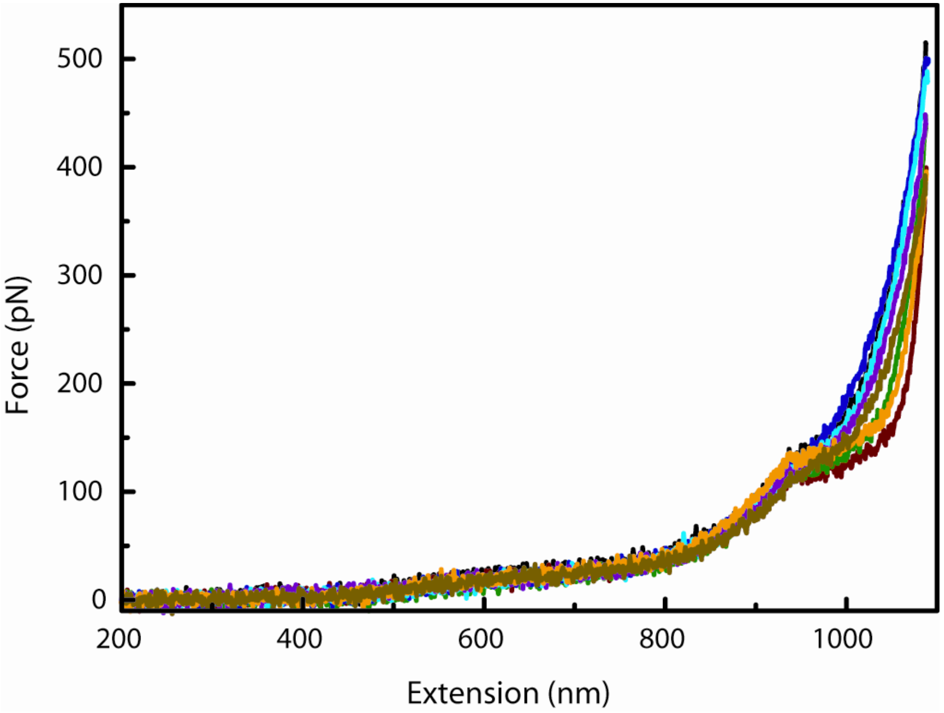
Forward and reverse takes different pathways

Multiple Pathways of PolydA Unstacking



Occasional hopping between two pathways

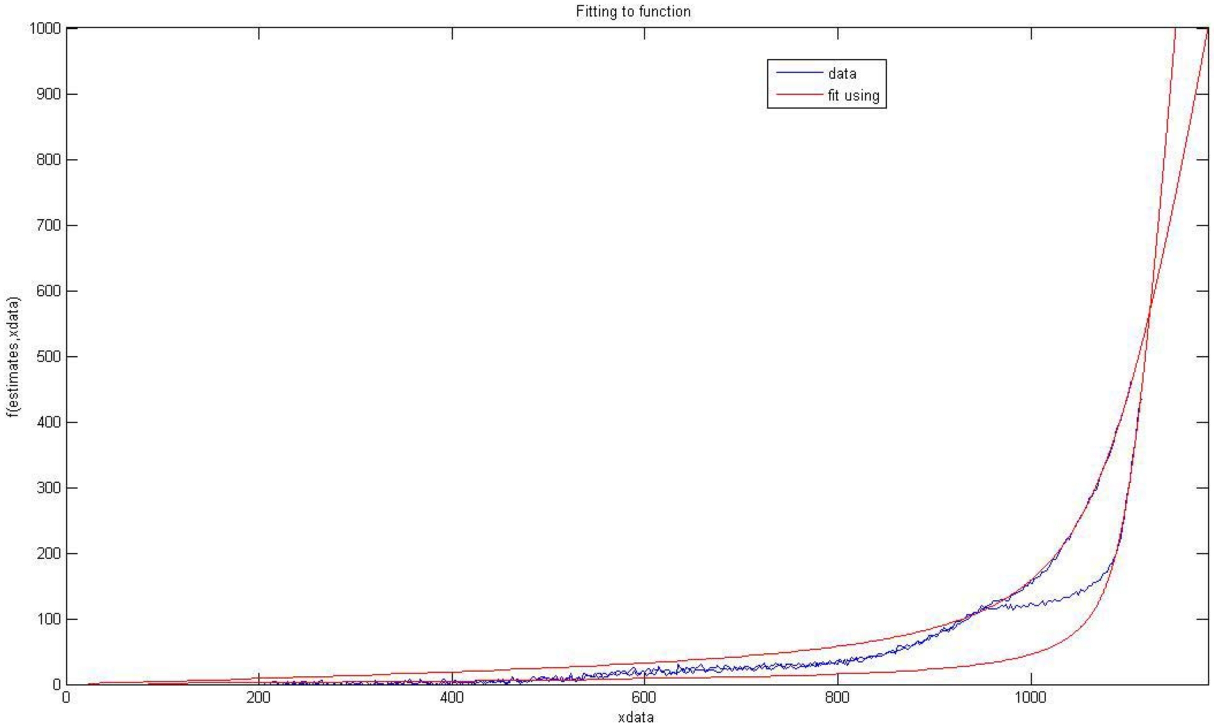
Multiple Stacking Pathways



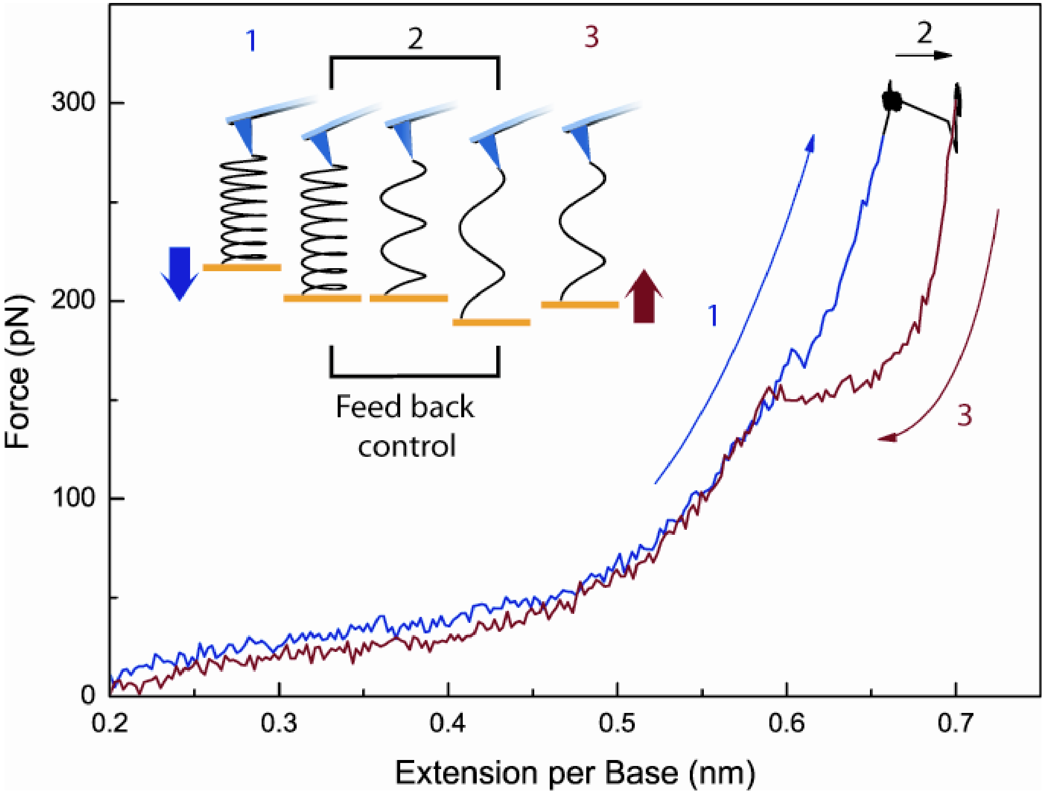
Different persistence length

Two Pathways Intersect

- The two pathways intersects at 600 pN, and the high energy pathway becomes the low energy pathway.

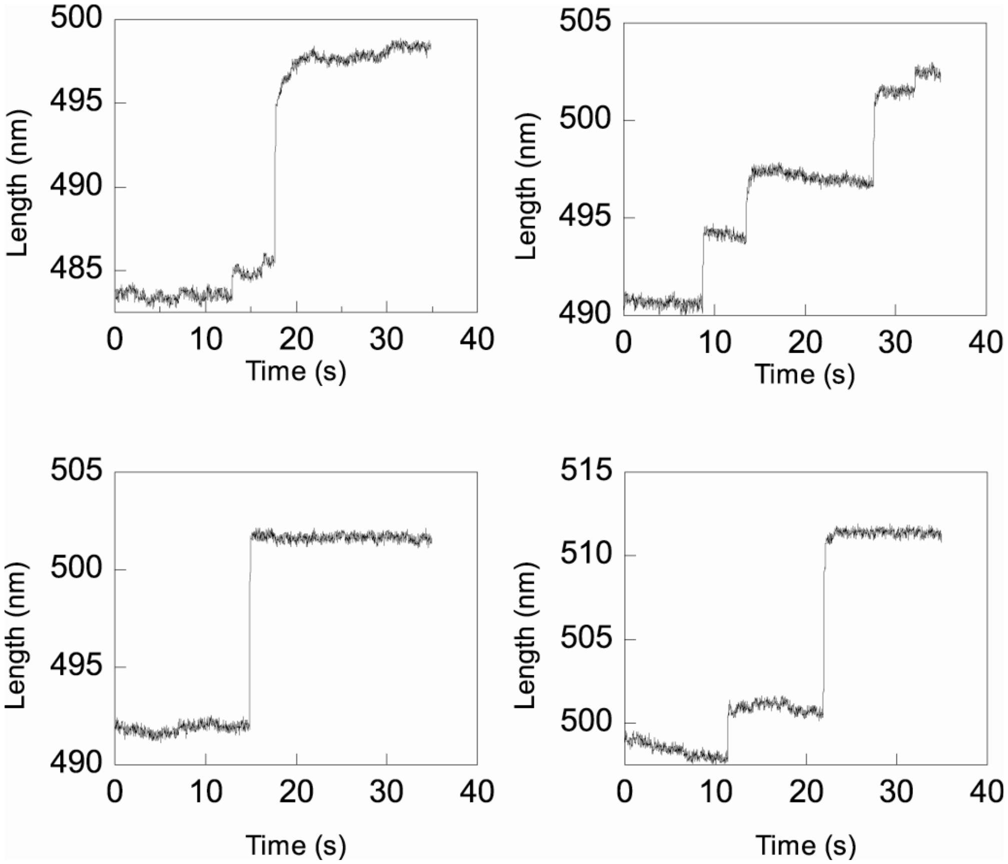


Constant Force Measurements



ssDNA was pulled to 300 pN and kept at constant force

Constant Force Measurements



- Cooperative transitions?
- Ground state configurations?
- dsDNA unzipping metastable states? *Danilowica et.al. PNAS 100, 1694 (2003).*

Conclusion I: Single-Stranded DNA

- PolydA mechanical overstretching via two pathways separated by a barrier
- Flipping of the backbone component is thermodynamically favored while direct elongation of the base distance is kinetically favored under certain environmental conditions
- The ground state conformation of polydA may be different from other ssDNA

Melting of dsDNA

DNA Based Nanosensors

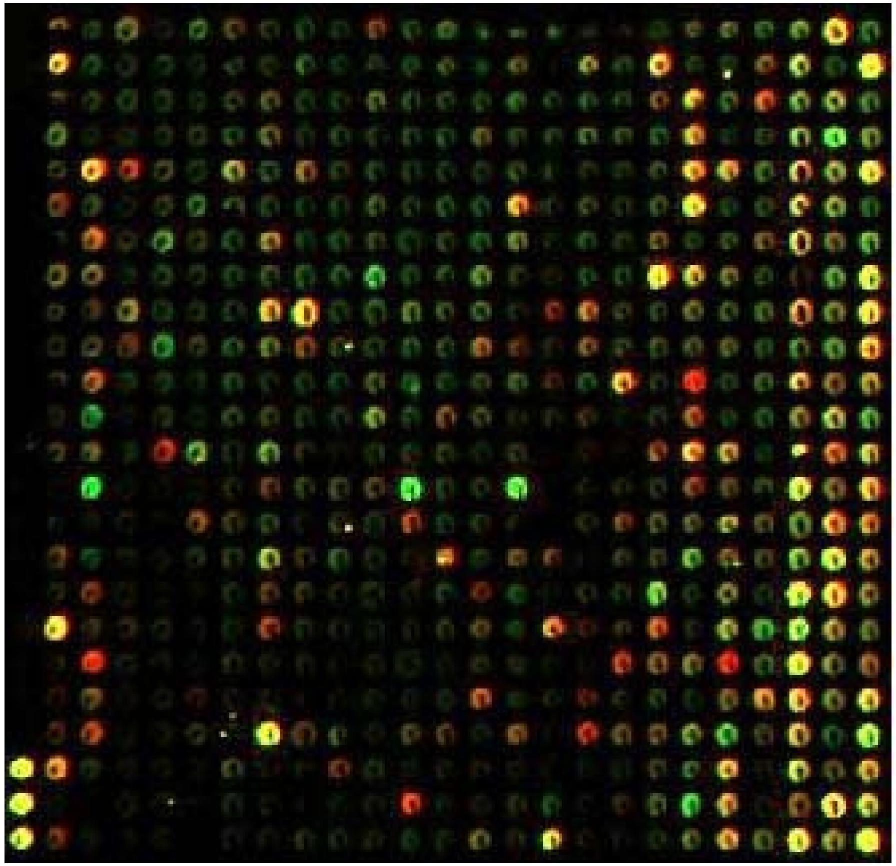
- Colloidal gold covered with oligonucleotide for DNA detection

  Mirkin et. al. Nature 382 (1996)

  Jin, Wu, Li, Mirkin, and Schatz, J. Amer. Chem. Soc. 125,1643 (2003)
- Used to detect Anthrax toxin
- An alternative technology to DNA microarray
- Understanding surface-bound DNA interactions

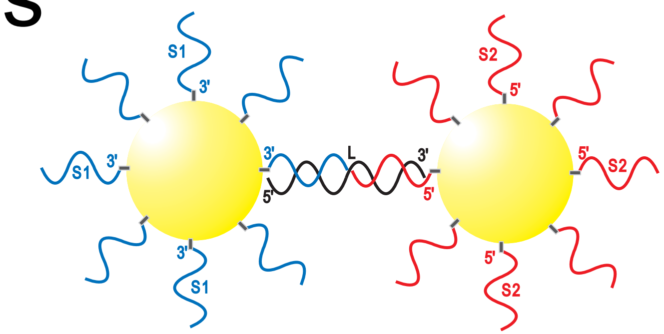
DNA Microarray

- DNA sensor
- Gene discovery
- Disease diagnosis
- Drug discovery



DNA-Linked Gold Nanoparticles

- Gold nanoparticle capped with ssDNA complementary to target (linker) ssDNA
- Probe particles self-assemble upon mixing with proper target DNA
- Color change upon phase transition
- New class of complex fluids



Sample Preparation

- Thiol modified DNA synthesis
- DNA-gold conjugation
- Excess DNA removal
- Target and probe DNA hybridization
- Aggregation kinetics and melting monitored by optical spectroscopy

Phase Transition of DNA-Linked Gold Nanoparticles

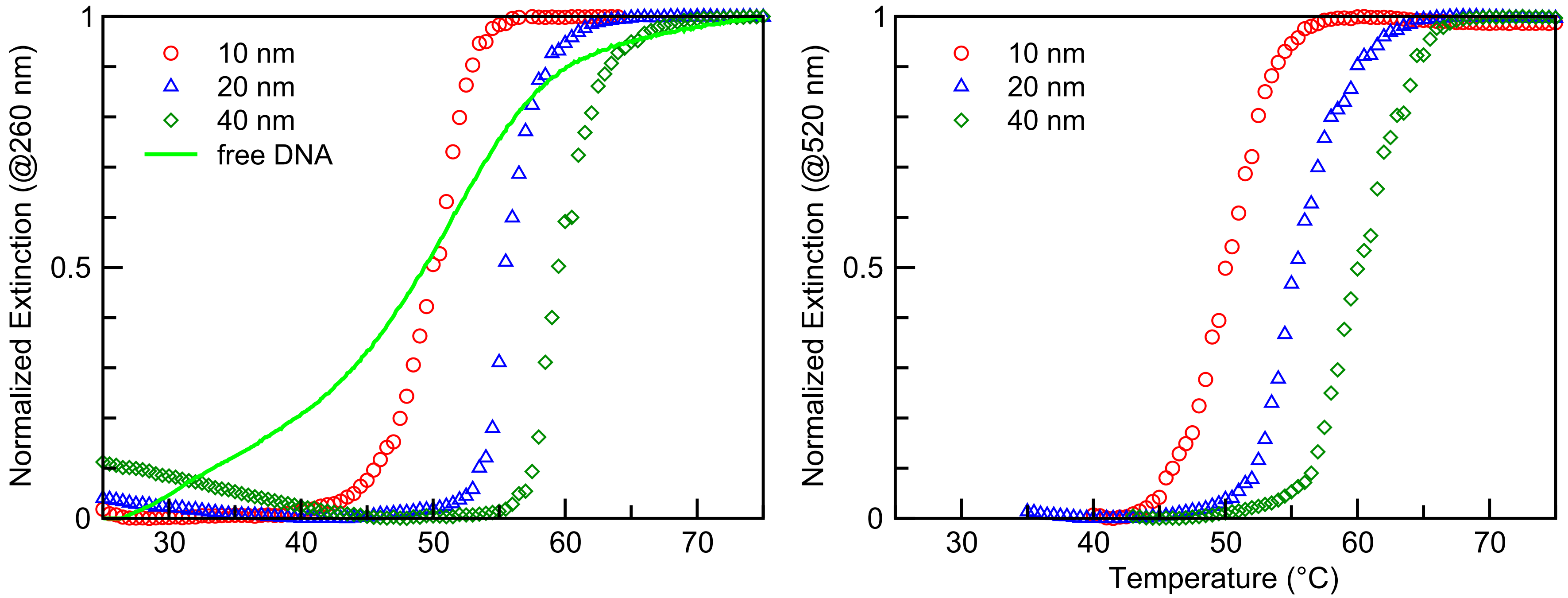
- Unique phase diagram
- Mapping microscopic DNA sequences onto the macroscopic phase behavior of colloids

  Lukatsky and Frenkel, Phys. Rev. Lett. 92, 068302 (2004)
- Optical properties and cluster aggregation thermodynamics and kinetics.

  Storhoff et. al., J. Amer. Chem. Soc. 122, 4640 (2000)

  Park and Stroud, Phys. Rev. B 68, 224201 (2003)

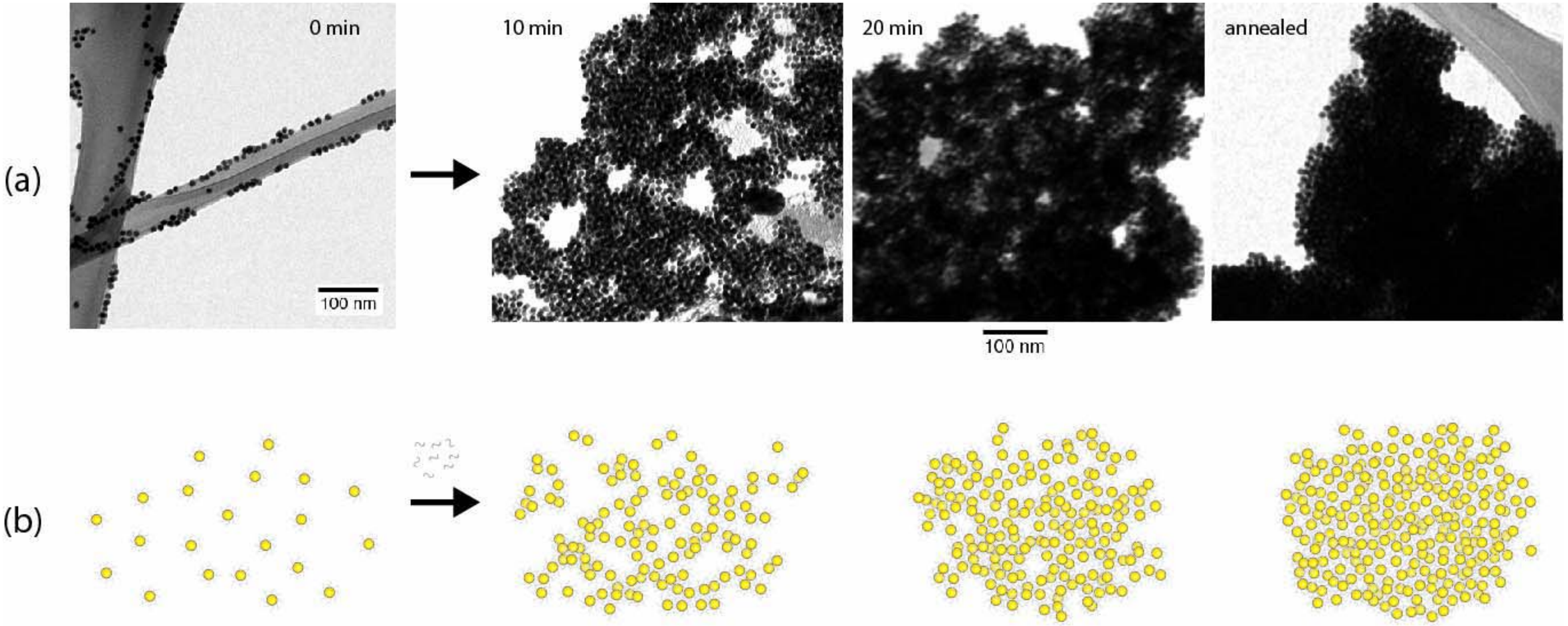
Melting Curves



Melting curves for gold particles and DNA are similar

Sun, Harris, and Kiang, Physica A, 354, 1 (2005)

Structural Phase Transition



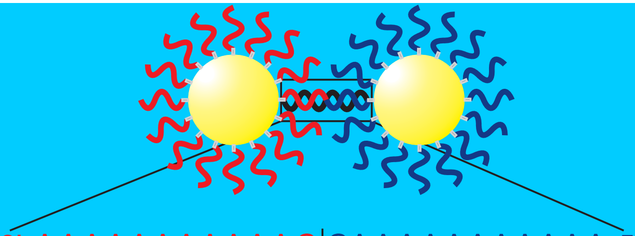
Amorphous structure

Sun, Harris, and Kiang, Physica A, 354, 1 (2005)

Using Simple DNA Sequences

- Eliminate sequence dependent phase transition properties
- Smooth and reproducible melting curves resulting in more accurate T_m determination
- Well-defined variables for isolating key effects
- Designing DNA-gold nanoparticles with specific interaction strength

Experimental Design



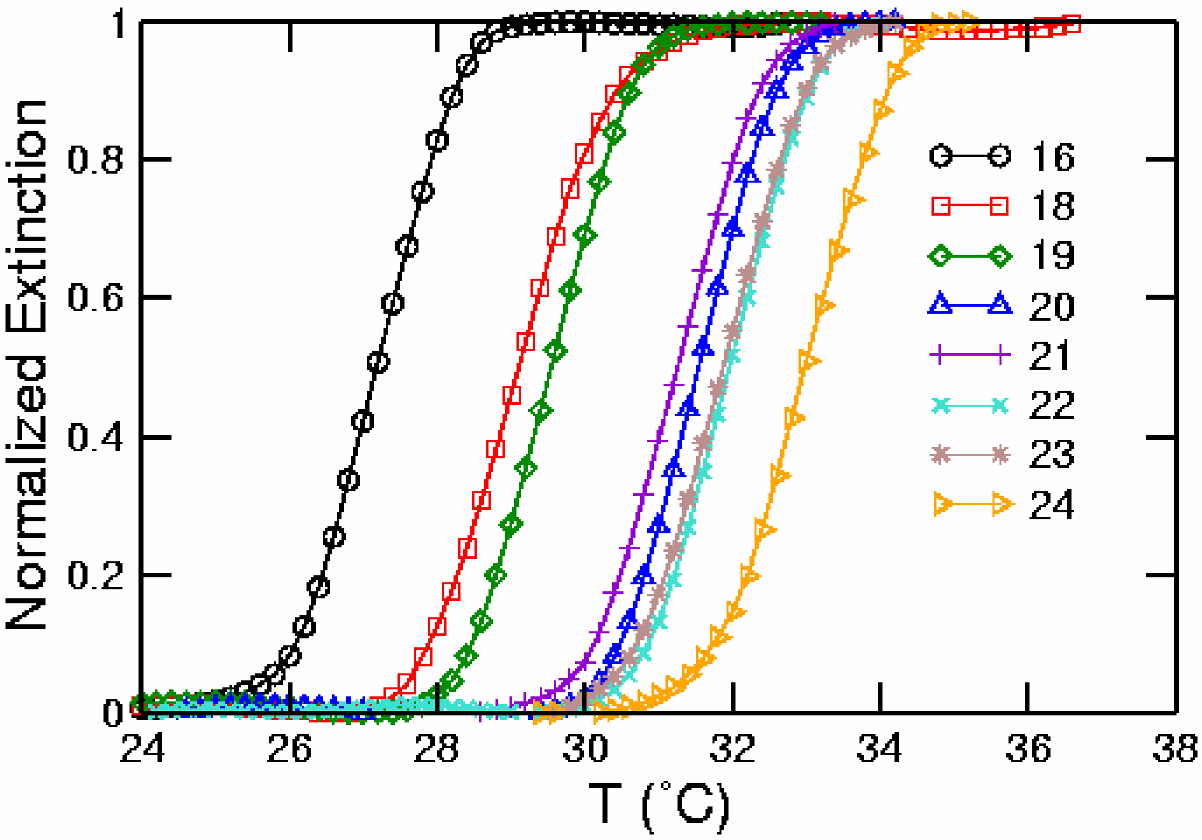
(a)

3'-AAAAAAAAAAAG|GAAAAAAAAAAA-5'	Probes
5'-TTTTTTTTTTTCCTTTTTTTTTTT-3'	Linker (12-12)
5'-TTTTTTTTTTTCCTTTTTTTTTT-3'	Linker (12-11)
5'-TTTTTTTTTTCCTTTTTTTTTT-3'	Linker (11-11)
5'-TTTTTTTTTTCCTTTTTTTTT-3'	Linker (11-10)
5'-TTTTTTTTTCCTTTTTTTTT-3'	Linker (10-10)
5'-TTTTTTTTTCCTTTTTTTT-3'	Linker (10-9)
5'-TTTTTTTTCCTTTTTTTT-3'	Linker (9-9)
5'-TTTTTTTCCTTTTTTT-3'	Linker (8-8)

(b)

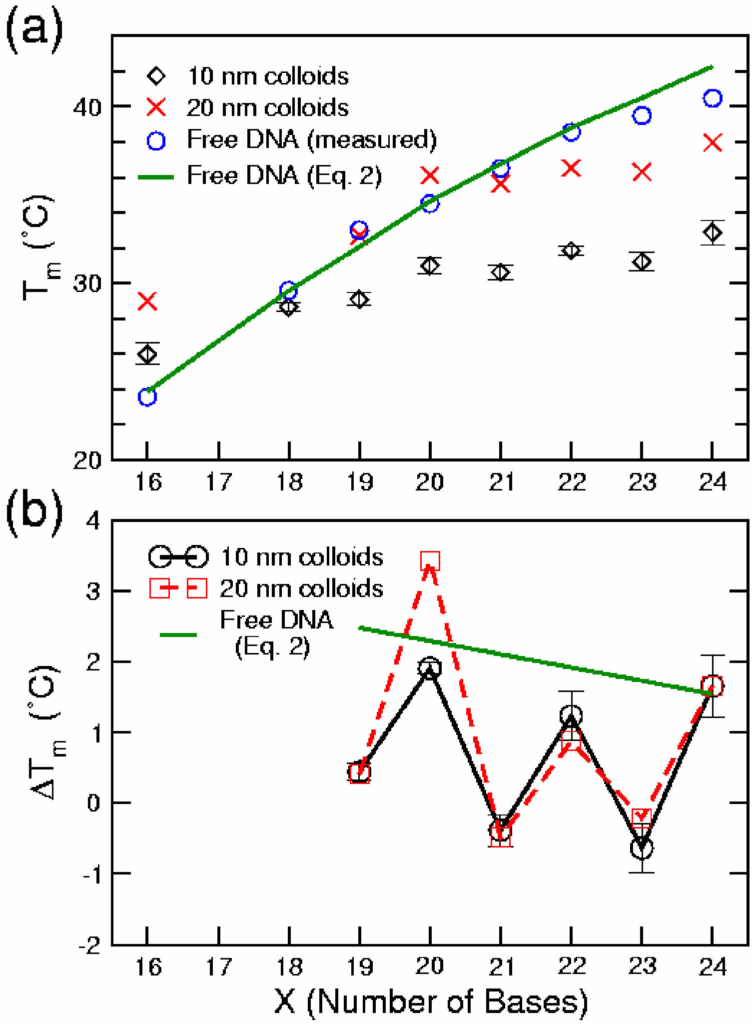
3'-AAAAAAAAAAAG|GAAAAAAAAAAA-5'	Probes
5'-TTTTTTTTTTCCTTTTTTTTTT-3'	Linker (11-11)
5'-TTTTTTTTTTTCCTTTTTTTTT-3'	Linker (12-10)
5'-TTTTTTTTTCCTTTTTTTTT-3'	Linker (10-10)
5'-TTTTTTTTTTCCTTTTTTTT-3'	Linker (11-9)
5'-TTTTTTTTTTTCCTTTTTTT-3'	Linker (12-8)

Effect of DNA Linker Length

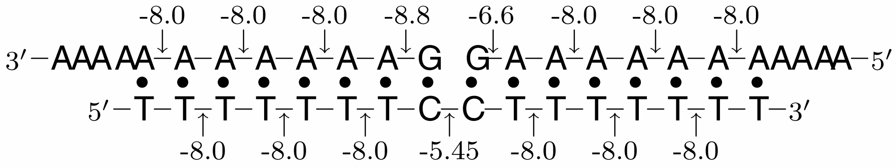


Harris and Kiang, Phys. Rev. Lett., 95, 0461101 (2005)

Effect of Disorder



$$T_m = \left(\frac{\Delta H^0 + 3.4\ \frac{\text{kcal}}{\text{mol}}}{\Delta S^0 - R\ln(\frac{1}{[\text{DNA}]})} \right) + 16.6\log_{10}([\text{Na}^+])$$



$5' : \Delta\text{H}^0 = 6(-8.0) + 1(-8.8) + 1(-5.45) = -62.25\ (\text{kcal/mol})$

$3' : \Delta\text{H}^0 = 6(-8.0) + 1(-6.6) + 1(-5.45) = -60.05\ (\text{kcal/mol})$

Disorder: Asymmetric Connection Energy

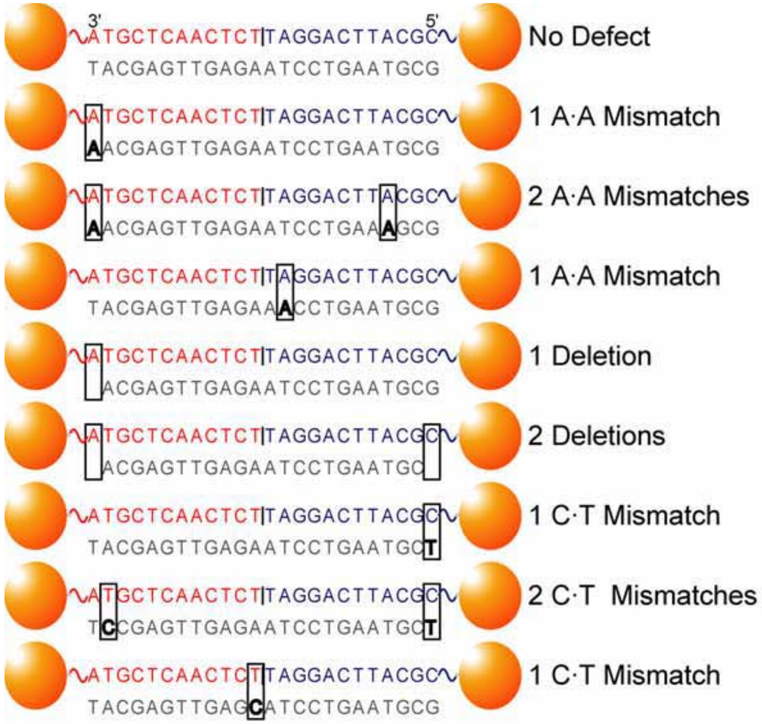
- In free DNA, T_m increases linearly with number of linker DNA bases
- Odd number of linker DNA bases results in lower T_m than expected in the nanoparticle systems

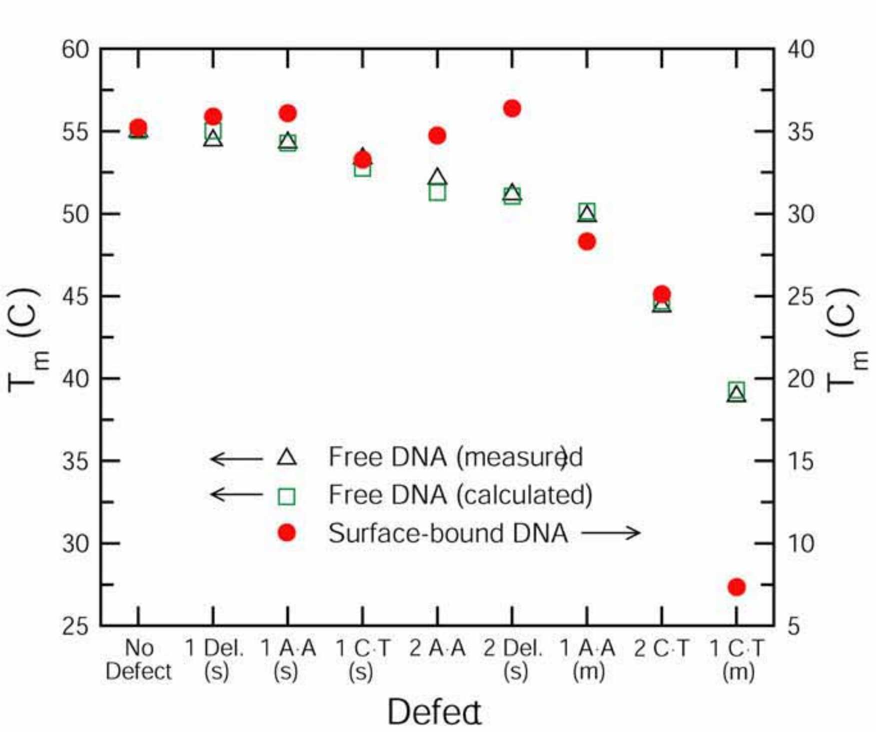
Harris and Kiang, Phys. Rev. Lett. 95, 0461101 (2005)

Mismatches and Deletions

- Present in DNA-linked gold nanoparticle system and DNA microarray
- Introducing error in DNA data
- Unexpected melting behavior
- Critical in interpreting data but poorly understood

T_m Trends in Bound vs Free DNA





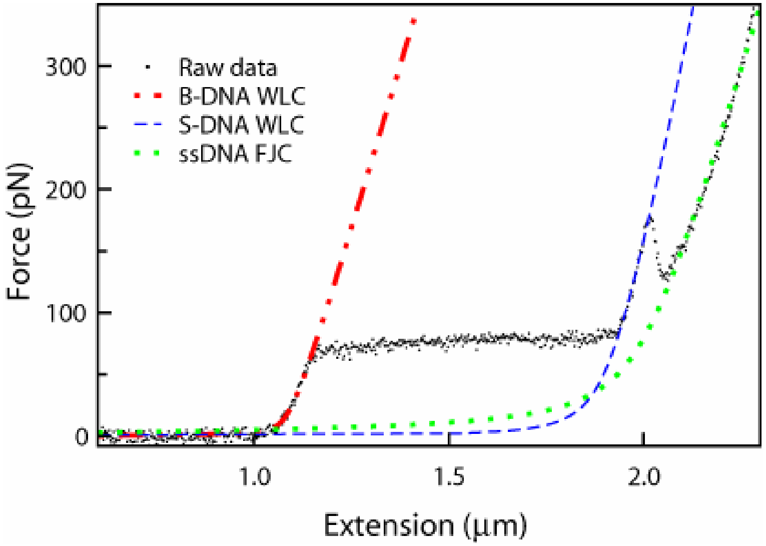
Defects may increase melting point

Harris and Kiang, J. Phys. Chem. B, 110, 16393 (2006)

Defects: Can Increase T_m

- Different from free DNA
- May increase melting temperature T_m
- Mismatches and deletions on or near surfaces are likely to increase T_m
- AA mismatches usually increase T_m, while CT mismatches decrease T_m
- Depending on factors such as base, sequence, and location
- May be used to increase detection sensitivity

Mechanical Melting of Double-Stranded DNA



Extensible Worm-like-chain (WLC)

$$x = b_{\mathrm{ds}}\left[1 - \frac{1}{\sqrt{4\beta P_{\mathrm{ds}} F}} + \frac{F}{K_{\mathrm{ds}}}\right]$$

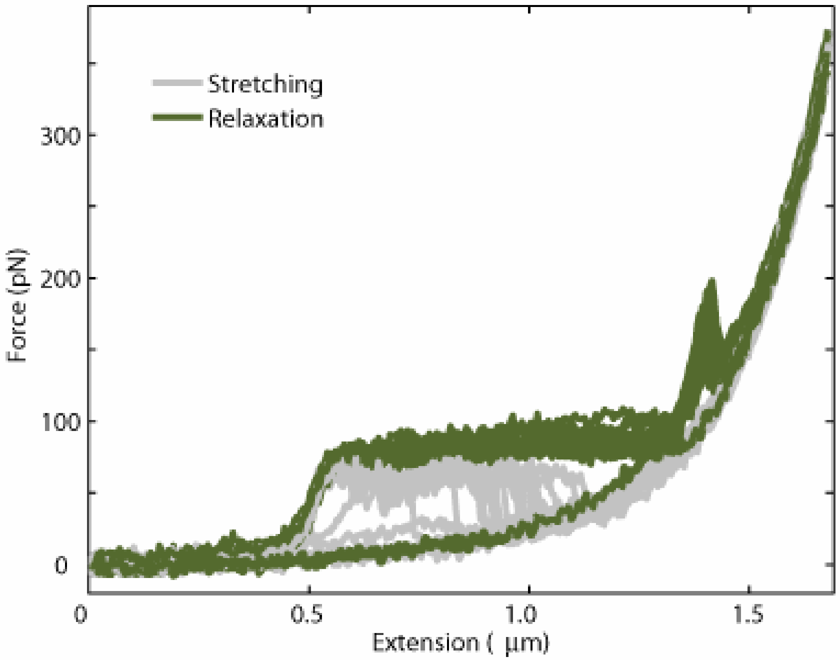
Extensible Freely-Jointed-Chain (FJC)

$$x = b_{\mathrm{ss}}\left[\coth(2\beta P_{\mathrm{ss}} F) - \frac{1}{2\beta P_{\mathrm{ss}} F}\right]\left[1 + \frac{F}{K_{\mathrm{ss}}}\right]$$

	P persistence Length (nm)	*b* contour length (nm)	*K* stretch modulus (pN)
B-DNA	50	1100	1200
S-DNA	10	2000	3200
ssDNA	0.75	2000	2200

Calderon et. al., J. Phys.: Condens. Matter 21 (2009) 034114.

B-S Transition of *dsDNA*



- Repeated stretch/relaxation cycle reproducible.
- Significant hysteresis during ssDNA relaxation cycle.

Summary II

- DNA-linked gold nanoparticle assemblies represents a new class of complex fluids, with tunable interaction between particles
- Introducing disorder and defects to the system results in melting temperature changes not explainable with free DNA thermodynamics
- Mechanical melting of dsDNA has a well-defined intermediate state

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