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
- Overstretching 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 overstretched transitions
• Elasticity of overstretched ssDNA
PolydA Exhibit Unique Stacking Behavior

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.
ssDNA was pulled to 300 pN and kept at constant force
• 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
  

- 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
  

- 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

Structural Phase Transition

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'-AAAAAAAAAAAAAG|GAAAAAAAGAAAAA-5'  
5'-TTTTTTTTTTTTTCTTTTTTTTTTTTTT-3'  Probes  
5'-TTTTTTTTTTTTTCTTTTTTTTTTTTTT-3'  Linker (12-12)  
5'-TTTTTTTTTTTTTCTTTTTTTTTTTTTT-3'  Linker (12-11)  
5'-TTTTTTTTTTTTTCTTTTTTTTTTTTTT-3'  Linker (11-11)  
5'-TTTTTTTTTTTTTCTTTTTTTTTTTTTT-3'  Linker (11-10)  
5'-TTTTTTTTTTTTTCTTTTTTTTTTTTTT-3'  Linker (10-10)  
5'-TTTTTTTTTTTTTCTTTTTTTTTTTTTT-3'  Linker (10-9)  
5'-TTTTTTTTTTTTTCTTTTTTTTTTTTTT-3'  Linker (9-9)  
5'-TTTTTTTTTTTTTCTTTTTTTTTTTTTT-3'  Linker (8-8)

(b) 3'-AAAAAAAAAAAAAG|GAAAAAAAGAAAAA-5'  
5'-TTTTTTTTTTTTTCTTTTTTTTTTTTTT-3'  Probes  
5'-TTTTTTTTTTTTTCTTTTTTTTTTTTTT-3'  Linker (11-11)  
5'-TTTTTTTTTTTTTCTTTTTTTTTTTTTT-3'  Linker (12-10)  
5'-TTTTTTTTTTTTTCTTTTTTTTTTTTTT-3'  Linker (10-10)  
5'-TTTTTTTTTTTTTCTTTTTTTTTTTTTT-3'  Linker (11-9)  
5'-TTTTTTTTTTTTTCTTTTTTTTTTTTTT-3'  Linker (12-8)
Effect of DNA Linker Length

Effect of Disorder

\[ T_m = \left( \frac{\Delta H^0 + 3.4 \text{ kcal/mol}}{\Delta S^0 - R \ln\left(\frac{1}{[\text{DNA}]^2}\right)} \right) + 16.6 \log_{10}(\text{[Na}^+]) \]

**Diagram a:**
- 10 nm colloids
- 20 nm colloids
- Free DNA (measured)
- Free DNA (Eq. 2)

**Diagram b:**
- 10 nm colloids
- 20 nm colloids
- Free DNA (Eq. 2)

DNA sequences:
- 3': AAAAAA A A A A A A A A
- 5': T T T T T T T T C T T T T T T T T

- Substitution:
  - 5' : \( \Delta H^0 = 6(-8.0) + 1(-8.8) + 1(-5.45) = -62.25 \) (kcal/mol)
  - 3' : \( \Delta H^0 = 6(-8.0) + 1(-6.6) + 1(-5.45) = -60.05 \) (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

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)

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

Extensible Freely-Jointed-Chain (FJC)

\[ x = b_{ss} \left[ \coth(2\beta_{ps} P_{ss} F) - \frac{1}{2\beta_p P_{ss} F} \right] \left[ 1 + \frac{F}{K_{ss}} \right] \]

<table>
<thead>
<tr>
<th></th>
<th>$P$ persistence Length (nm)</th>
<th>$b$ contour length (nm)</th>
<th>$K$ stretch modulus (pN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-DNA</td>
<td>50</td>
<td>1100</td>
<td>1200</td>
</tr>
<tr>
<td>S-DNA</td>
<td>10</td>
<td>2000</td>
<td>3200</td>
</tr>
<tr>
<td>ssDNA</td>
<td>0.75</td>
<td>2000</td>
<td>2200</td>
</tr>
</tbody>
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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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