Untangling the Dynamics of Entangled DNA

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Funding:
Research Corporation
AFOSR Young Investigator Award
Soft Squishy Matter is amazing, complex & all around us

Fascinating, multifunctional viscoelastic properties

Comprised of polymers

What’s happening on a molecular level to give rise to such dynamic, complex behavior?

Macroscopic Viscoelasticity

10^{23} Entangled Polymers

Molecular-level Dynamics & Interactions
DNA is a model polymer to answer this question

**DNA Replication**

- Homogeneous samples equal length

**Enzymes can precisely control topology**

- Supercoiled
- Circular
- Ring
- Linear

**Direct single-molecule visualization**

- 45 kbp (15 μm)

**Direct molecular-level force measurement**

- 12 μm
Reptation Tube theory describes basic entangled polymer dynamics but not exact.

Many-body problem

one body, mean field
Confining Tube Radius
only parameter needed

Curve-linear diffusion
along tube contour

Classical reptation tube theory unable to accurately account for:

mobile constraints

Different topologies

response to large nonlinear deformation
Non-ideal polymer systems are still poorly understood

Multiple new theories proposed
Conflicting experimental & theoretical results

We need experiments that can directly probe the molecular dynamics and interactions that give rise to the poorly understood material properties

Classical reptation tube theory unable to accurately account for:

- Mobile constraints
- Different topologies
- Response to large nonlinear deformation
How we probe molecular-level DNA dynamics and what we find

Linear & Ring DNA Blends

Molecular Crowding

Fluorescence Microscopy Particle Tracking

Nonlinear Viscoelasticity

Optical Tweezers Microrheology

Noncontinuum Effects
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Fluorescence Microscopy Particle Tracking
How we track single DNA molecules to directly measure diffusion

Label DNA with fluorescent dye

Mix with unlabeled DNA or crowder

Directly observe Brownian motion of single DNA molecules

**Track** Center of Mass of DNA

**Calculate** Diffusion Coefficient

\[ \langle (\Delta x)^2 \rangle = 2D_t \]
How we probe molecular-level DNA dynamics and what we find

Linear & Ring DNA Blends

Fluorescence Microscopy Particle Tracking
Ring DNA is drastically slowed by linear DNA

Linear DNA entanglements force Ring DNA to diffuse via constraint release

Constraint release is much slower than reptation

Linear DNA slows down then speeds up as linear fraction increases

linear DNA Diffusion vs. blend fraction

Previously unpredicted, unobserved nonmonotonic diffusion dependence on blend fraction

Simulations reveal molecular mechanisms underlying nonmonotonic diffusion

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Lattice bond-fluctuation model simulations match experimental data

Simulations reveal molecular mechanisms underlying nonmonotonic diffusion

Non-monotonicity: 2\textsuperscript{nd} order effect of entangling rings being slowed by increased threading events

Lattice bond-fluctuation model simulations match experimental data

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Molecular Crowding

Fluorescence Microscopy Particle Tracking
Macromolecular crowding produces complex inhomogeneous environments

Crowded Environments have macromolecules of different sizes, properties, structures

Crowding in cells plays a dominant role in nucleic acid and protein mobility, conformation and function

0-40% dextran solutions mimic cellular crowding conditions

Diffusion Experiment Parameters
Crowder size plays a dominant role in DNA diffusion

DNA diffusion in dextran solutions

- Smaller crowders: slowing effect saturates at ~20%
- Larger crowders: DNA mobility is exponentially reduced by increased crowding

DNA size plays minimal role in its mobility not previously reported or predicted

- Larger crowder is 7x slower than smaller crowder
- Less mobile crowders cause extreme ~300x DNA mobility reduction

Mathematical formula:

\[ \frac{D}{D_0} \sim e^{-C/C^*} \]

\[ C^* = 7.0 \]
Crowding induces elongation of DNA from random coil.

Crowding forces DNA into lower entropy state driven by entropy maximization of crowders.

- Short DNA; large crowder
- Long DNA; small crowder
- Long DNA; large crowder

Entropic effects in cell necessary for:
- DNA-protein binding
- Chromosomal compaction
- Protein folding
Major and minor axis lengths quantify DNA elongation

Elongation: $R_{\text{max}}/R_{\text{min}} - 1$

Distribution of elongations sampled by crowded DNA

- Short DNA; large crowder
- Long DNA; small crowder
- Long DNA; large crowder
Elongation is governed by size of DNA and crowder

Short DNA:
- fewer conformational states when crowded

Less elongation induced by small crowder
DNA coil penetration

Large crowder:
- 67% elongation of long DNA
Changes in major axis length with time quantify state fluctuations

Fluctuations of DNA Elongation in Time

Fluctuation Length: \(<R_{max}(t) - R_{max}(0)\>

Crowding slows fluctuation rate
Crowding can increase or reduce DNA state fluctuations depending on DNA size

Short DNA fluctuates less
Reduced entropy

Long DNA fluctuates more
Offsets extreme elongation
How we probe molecular-level DNA dynamics and what we find

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Optical Tweezers Microrheology
Macroscopic Strain \( (\gamma) \) exerted

Resultant Stress \( (\sigma) \) on material measured
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Nonlinear Viscoelasticity

Optical Tweezers Microrheology
The response of entangled polymers to large deformations is complex and not well understood.

**Small strain amplitudes = linear viscoelastic response**

- No disruption of entanglements
- No chain stretching

**Large strain amplitudes & rates = nonlinear viscoelastic response**

- Classical tube theory cannot explain bulk experimental data
- New proposed theories remain untested

Classical tube theory
Describes data well
We measure the molecular-level DNA response to large strains

1) Trap bead

2) Pull bead 30 µm through DNA

2) Measure force DNA exerts

3) Hold bead in trap for fixed time after strain

4) Release bead from trap

4) Track bead “recoil”

Chapman, Robertson-Anderson; Physical Review Letters (2014)
We measure the molecular-level DNA response to large strains

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10 Pulling rates:
~3 – 126x DNA disentanglement rate
Wiesenberg No. (Wi) = 3 - 126

3 DNA concentrations:
~0.5 – 2x entanglement concentration

Chapman, Robertson-Anderson; Physical Review Letters (2014)
The local DNA response displays key nonlinear features explained by non-classical entanglements

- **Stress Stiffening**
- **Stress Softening**
- **Viscous behavior**
- **Nonlinear viscosity thinning for $Wi > 20$**
- **Elastic Yielding Strain**
- **$Wi > 20$: Nonlinear tube softening entanglement loss**

Chapman, Robertson-Anderson; Physical Review Letters (2014)
Free motion of bead following strain characterizes stored entanglement elasticity

3) Hold bead in trap for fixed time after strain

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Bead Recoil distance vs. time

Chapman, Robertson-Anderson; Physical Review Letters (2014)
Recoil shows non-classical tube dilation in agreement with recent nonlinear theory

Bead Recoil distance vs. time

Maximum Recoil distance vs Strain Rate

Exponential recoil to maximum recoil distance

Recoil distance comparable to Entanglement tube diameter

Agreement with non-classical entanglement theory [Sussman, Schweizer, 2013]

Strain-induced rate-dependent tube dilation

Tube potential directly coupled to external stress

Chapman, Robertson-Anderson; Physical Review Letters (2014)
Recoil decay rate shows crossover to non-classical entanglement tube healing and relaxation

1. Fast tube retraction
2. Classical tube relaxation
3. Tube healing to equilibrium size

Wi < 20
Single classical tube relaxation

Tube dilates with increasing strain rate

Multimode tube relaxation and power-law tube healing
Agreement with non-classical entanglement theory [Sussman, Schweizer, 2013]

Chapman, Robertson-Anderson; Physical Review Letters (2014)
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Optical Tweezers Microrheology
Optical Tweezers microrheology measures molecular-level dynamic moduli

**Microrheology**

**Oscillatory** Strain ($\gamma$) exerted
Oscillatory Stress ($\sigma$) measured

**Solid: Elastic**

$\sigma \sim \gamma$

**Fluid: Viscous**

$\sigma \sim \dot{\gamma}$

**Dynamic moduli** quantify amount of elasticity and viscosity

**Elastic/Storage Modulus**

$$G' = \frac{\sigma_0}{\gamma_0} \cos(\phi)$$

**Viscous/Loss Modulus**

$$G'' = \frac{\sigma_0}{\gamma_0} \sin(\phi)$$
The size of the probing microsphere matters

Comparison of macro- and microrheology relies on **assumption**: material is treated as **continuum** on length scale of **probe**

Polymeric Fluids are **not continuum** materials
They have intrinsic **length scales**

What is the **Critical Size** for **microspheres** to probe full viscoelasticity of micro-environment?

- **Mesh size**: \(\sim 100 \text{ nm}\)
- **Tube diameter**: \(\sim 0.7 \mu \text{m}\)
- **Radius of Gyration**: \(\sim 0.3 - 1 \mu \text{m}\)
- **Persistence length**: \(\sim 50 \text{ nm}\)
The size of the probing microsphere matters

Entanglements dominate DNA response with large probes
Continuum limit reached for probes >2 μm

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Threshold Probe Radius: $R_{th} \approx 2 \, \mu m$

Threshold probe size is larger than many probes used in microrheology experiments!

Critical probe size is 3-5x the theoretical tube radius

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Threshold Probe Radius $R_{th} \approx 5d_T$
Agreement with underutilized prediction

Molecular-level experiments reveal complex DNA dynamics in entangled & crowded systems

Non-monotonic diffusion effects in linear-ring blends

Crowding-induced mobility reduction & entropic elongation

Crossover to nonlinear viscoelasticity driven by non-classical entanglements

Critical Continuum limit bead size of ~3-5x the entanglement tube diameter
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