Untangling the Dynamics of Entangled Biopolymers

Rae M. Robertson-Anderson

University of San Diego
COLLEGE OF ARTS AND SCIENCES
Department of Physics and Biophysics

NSF CAREER Award
AIR FORCE OFFICE OF SCIENTIFIC RESEARCH
UNITED STATES AIR FORCE

MOORE FOUNDATION
RESEARCH CORPORATION FOR SCIENCE ADVANCEMENT

GORDON AND BETTY MOORE FOUNDATION
Soft Squishy Matter is amazing, complex & all around us

Fascinating, multifunctional \textit{viscoelastic} properties

Comprised of \textit{polymers}

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

\textbf{Macroscopic Viscoelasticity} \quad 10^{23} \text{ Entangled Polymers} \\
\textbf{Molecular-level Dynamics & Interactions}
Biopolymers are highly versatile and tunable

- **DNA: flexible**
  Persistence Length: 50 nm
  Typical length: 5 – 20 μm

- **Actin: semi-flexible**
  Persistence Length: ~17 μm
  Typical length: 5 – 20 μm

- **Microtubules: Rigid**
  Persistence Length: ~1 mm
  Typical length: 10 – 50 μm

Replication controls **length**

Enzymes control **topology**

Polymerization controls **length**

Crosslinking proteins control cytoskeleton network architecture
We can image single biopolymers & measure intermolecular forces

Fluorescence Microscopy & Molecular Tracking

Optical Tweezers Microrheology
We investigate macromolecular mechanics of entangled cytoskeleton networks. Linking molecular motion to stress propagation.

Actin mechanics during disassembly & reassembly.

Mesoscale mechanics of cytoskeleton composites.


Gurmessa, et al; PNAS (in preparation)


Ricketts, et al; Soft Matter (in preparation)
We investigate macromolecular mechanics of entangled cytoskeleton networks

Linking molecular motion to stress propagation

We track actin filament deformations induced by microscale strains

We map filament deformations throughout the network out to mesoscopic length scales.

Discretely label segments along entangled actin filaments.

Track segment displacements during and following microscale strain.
We couple microscale stress response to induced deformations of actin filaments.

Measure actin velocity distributions

Measure force filaments exert to resist strain
We link filament motion to microscale forces and mesoscale network deformations.

What macromolecular deformations lead to the resistive network force response?

How do induced deformations & stress propagate through the network?

Measure actin velocity distributions

Measure force filaments exert to resist strain
Stiffness of actin networks increases exponentially with degree of crosslinking.

Networks stiffen then soften to steady-state.

Without crosslinks, network yields to steady-state viscous regime ($K \approx 0$).

Stored elasticity increases exponentially with crosslinking ratio.

Critical crosslinker ratio $R$ for crosslinking to dominate response:

crosslinker length $l_c \approx \xi$.

Maximum stiffness

Steady-state stiffness

Time to reach viscous regime
Viscous dissipation is inhibited only when crosslinker length $\leq$ mesh size

Critical crosslinker ratio $R$ for crosslinking to dominate response:

crosslinker length $l_c \approx$ mesh size $\xi$

Steric crossings can slip past each other enabling "flow" and dissipation

Once most crossings are permanently linked, networks become elastic

![Diagram showing network structure and parameters](image)

### Graphs

- **C**: Maximum stiffness vs. $l_c$
- **D**: Steady-state elasticity vs. $l_c$
- **E**: Time to reach viscous regime vs. $l_c$
Filaments accelerate then retract from disentanglement & crosslinker unbinding

Constant rate strain yet filaments accelerate, decelerate, and retract

Filaments are stretched along strain path then release from stressed path and retract

Elastic retraction only possible when filaments are crosslinked
Filament deformation decays exponentially with distance from strain site

Critical distance from strain site $\approx$ persistence length

Elastic retraction only possible when filaments are crosslinked

Maximum deformation $>$ mesh size $>$ crosslinker length

Strain induces disentanglement and crosslinker unbinding

How does locally induced strain propagate through the network?
Crosslinking leads to partial elastic recovery + plasticity from unbinding and rebinding

Mechano-memory from crosslinking

Plasticity due to transient crosslinker unbinding and rebinding

Force relaxation exhibits distinct fast and slow exponential decays

Relaxation times and terminal force exponentially increase with crosslinking ratio

Filaments **elastically recoil** backwards despite **sustained elastic force**

Recoil increases linearly with crosslinking ratio
Crosslinking leads to partial elastic recovery + plasticity from unbinding and rebinding

How can force be maintained if most filaments release built-up stress?

How is stress distributed throughout the network?

Filaments elastically recoil backwards despite sustained elastic force

Recoil increases linearly with crosslinking ratio
Stress is distributed non-uniformly to discrete percolated stress fibers in the network

How can force be maintained if most filaments release built-up stress?

How is stress distributed throughout the network?

Conventional network theory: stress distributed uniformly through network

New theory & simulations:
stress is maintained in small fraction of filaments – percolating stress fibers
Majority of network can release stress and return to steady-state
We investigate macromolecular mechanics of entangled cytoskeleton networks.

Actin mechanics during disassembly & reassembly

Gurmessa, et al; PNAS (in preparation)
We measure the force response of disassembling and reassembling actin networks

Actin networks in cytoskeleton continuously disassemble and reassemble

Guramessa, et al; PNAS (in preparation)
We measure the force response of disassembling and reassembling actin networks.

\[ G' = \frac{F_0 \cos \Delta\delta}{x_0 6\pi \eta R} \]

\[ G'' = \frac{F_0 \sin \Delta\delta}{x_0 6\pi \eta R} \]

Elastic Modulus

Viscous Modulus

Gurmessa, et al; PNAS (in preparation)
During disassembly the elastic modulus exhibits two distinct exponential decays.

Elastic Modulus during Disassembly

Theoretical prediction for disassembly of actin of varying lengths

Initial actin lengths: 6 ± 4 μm

Filaments disassemble to monomers over 150 mins

Sharp transition from slow to fast decay ~90 mins

Initial slow decay: actin filaments depolymerize with network still intact

Fast decay: depolymerization once network is disassembled

Gurmessa, et al; *PNAS* (in preparation)
Slow elongation kinetics lead to linear increase in the elastic modulus during reassembly

Linear increase: filaments elongate to form network

Steady-state Plateau: fully-percolated network forms after ~90 mins

Slopes and Crossover Time governed by elongation rates

Filaments elongate at ~0.3 s⁻¹

Gurmessa, et al; PNAS (in preparation)
We investigate macromolecular mechanics of entangled cytoskeleton networks.

Mesoscale mechanics of cytoskeleton composites

We characterize the mesoscale mechanics of actin-microtubule composites

We use optical tweezers to induce fast mesoscale strains in composites.

We designed co-polymerized actin-microtubule composites with varying actin:tubulin ratios.

We measure the force that composites exert to resist the strain.
Viscoelastic composites are able to stress-stiffen and resist compressive buckling.

Microtubules support stress-stiffening by suppressing actin bending fluctuations.

Composites transition from elastic to viscous response during strain.

**Microtubules** increase resistive force.

**Actin** stabilizes microtubules against compressive buckling.

Mesh size mismatch between actin and microtubules enables tunable strength and uniformity

Microtubules produce:
- larger resistive forces
- More heterogeneous response

Strain induces microtubule buckling without actin network present

Actin mesh size < microtubule mesh size at a given molarity

Actin enables more uniform microscale force response of microtubule composites

Ricketts, et al; Soft Matter (in preparation)
Composites exhibit unique two-phase power-law force relaxation

Fast relaxation: bending fluctuations of actin filaments
Slow relaxation: diffusion of actin and microtubules out of deformed entanglements

Microtubules retard and suppress fast relaxation
Slow relaxation is independent of composite composition

Microscale measurements reveal complex molecular mechanics in cytoskeleton networks

Linking molecular motion to stress propagation

Nonlinear actin deformations lead to stiffening, yielding, and non-uniform stress propagation


Actin mechanics during disassembly & reassembly

Dis/Re-assembling actin networks undergo a sharp percolation phase transition

Gurmessa, et al; *PNAS* (in preparation)

Mesoscale mechanics of cytoskeleton composites

Actin-microtubule composites exhibit stress-stiffening, resilience, and uniformity

Here are the students, postdocs, and collaborators who actually did all the work!

**Linking molecular motion to stress propagation**


**Actin mechanics during disassembly & reassembly**

Gurmessa, et al; *PNAS* (in preparation)

**Mesoscale mechanics of cytoskeleton composites**