Biopolymer Network Mechanics , Part II

Boundary Effects, Limitations to Microrheology

Cytoskeletal Mechanics

Nonlinear Effects

Importance of Crosslinking

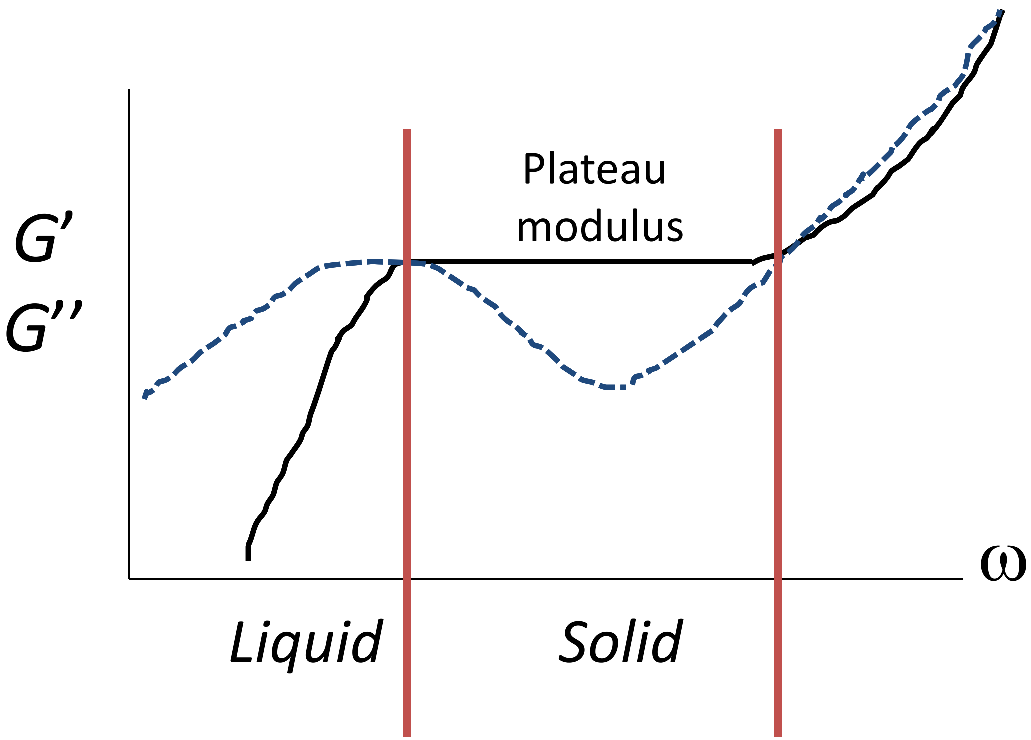
Extensions to Active Materials and Cells

Report back….

Anyone measure anything interesting?

Quick review…

$$\sigma = \gamma_0 \left[G' \sin(\omega t) + G'' \cos(\omega t) \right]$$



Crossovers point to interesting time scales

Conventional Rheometry: Summary

Advantages:

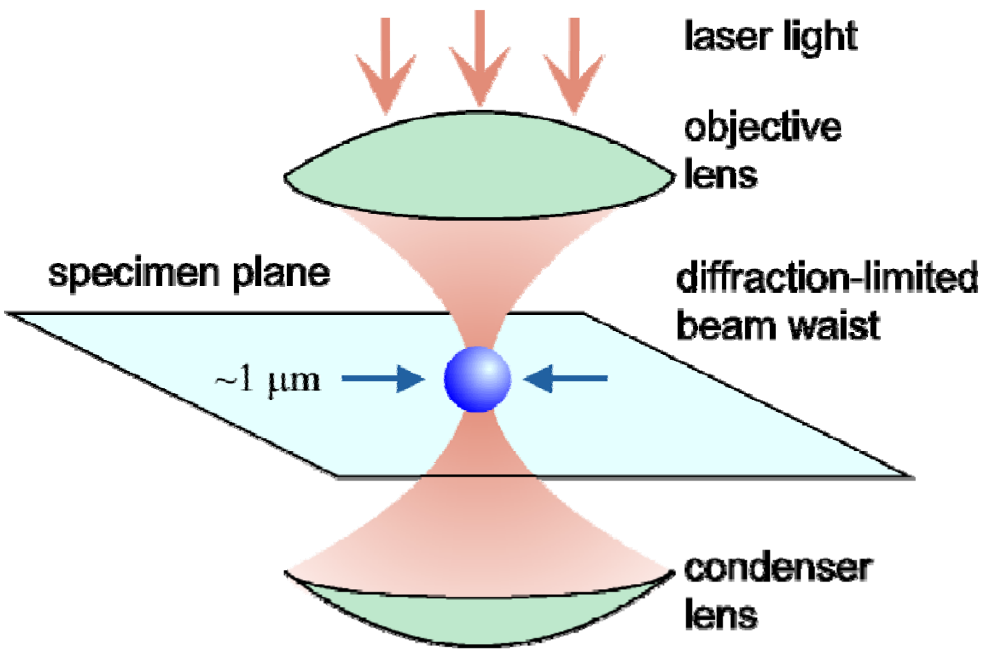
- Direct measurement
- Strain- and frequency-dependent measurements
- Probes average behavior
- Fairly easy, fast

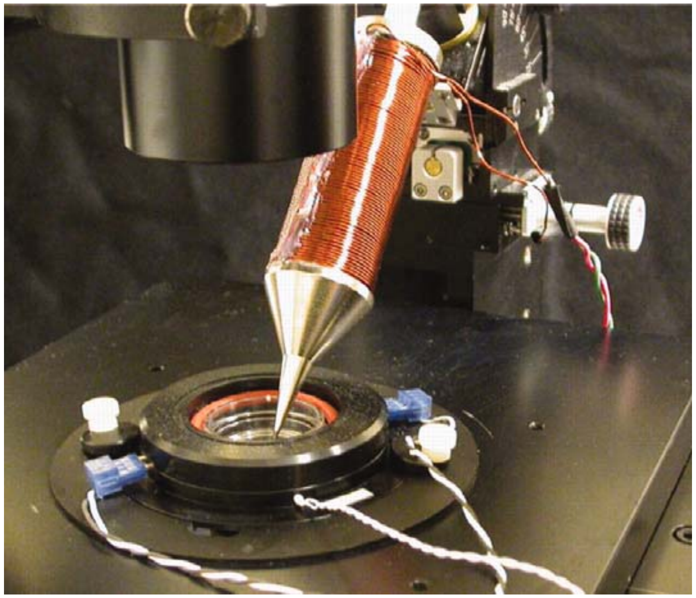
Disadvantages:

- Large sample volumes (>500 μL)
- Fragile materials can be damaged during loading
- Limited frequency range
- Difficult to study heterogeneous materials

Microrheology is a good alternative, especially for biomaterials

Active Methods: Material is locally deformed, and microscopic viscoelastic response recorded.

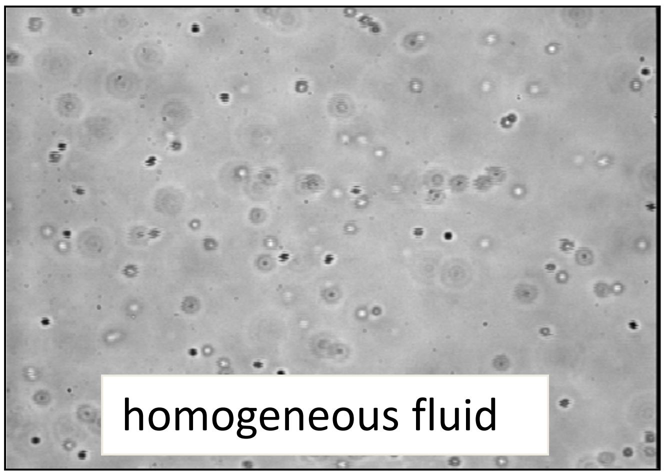




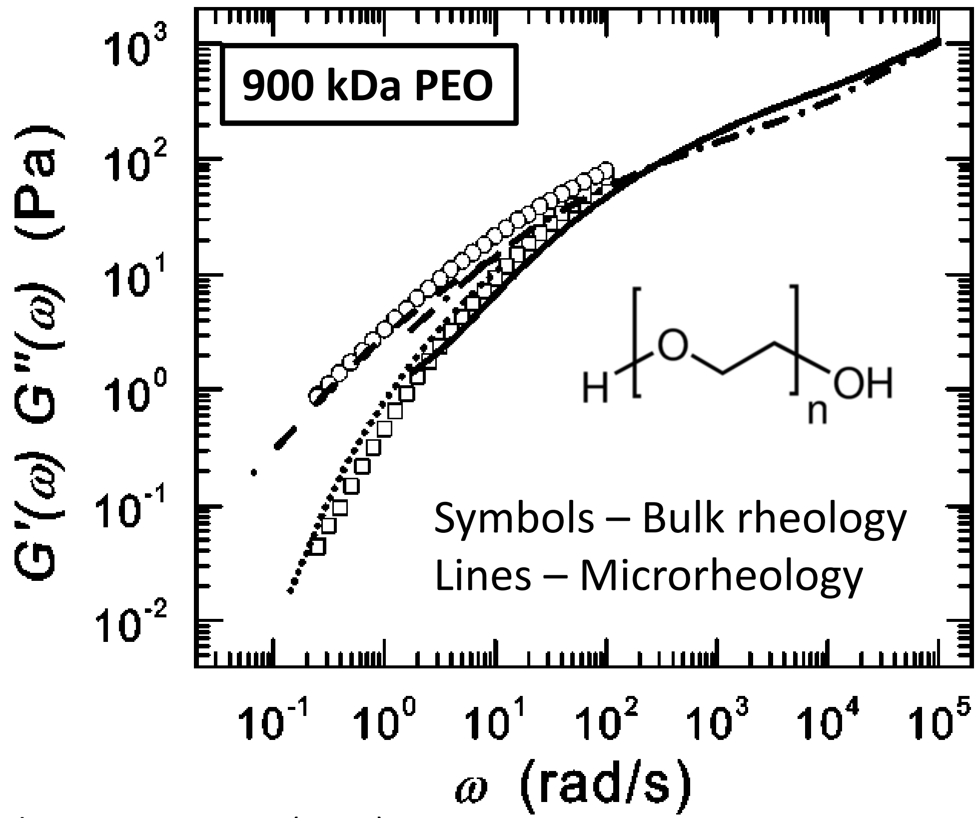
Huang H et al. *Am J Physiol Cell Physiol* (2004)

Microrheology is a good alternative, especially for biomaterials

Passive Methods: Tracer particles are embedded in a complex material, and their thermal displacements measured.

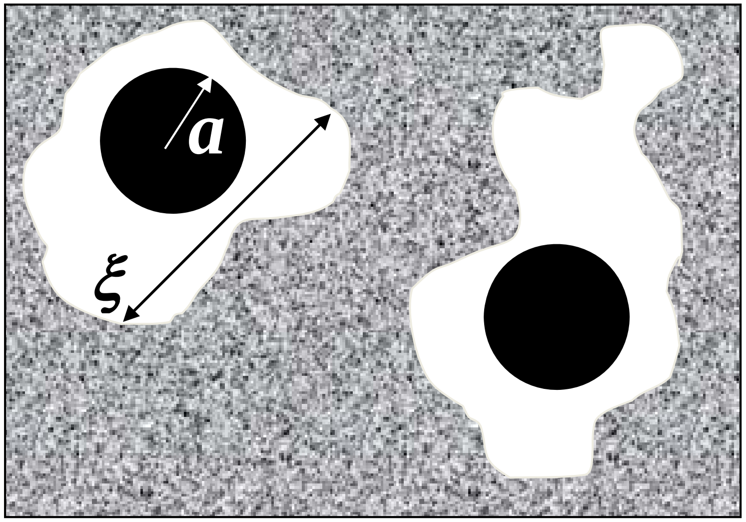


$$G(s)=\frac{k_BT}{3\pi as\langle\Delta x^2(s)\rangle}$$



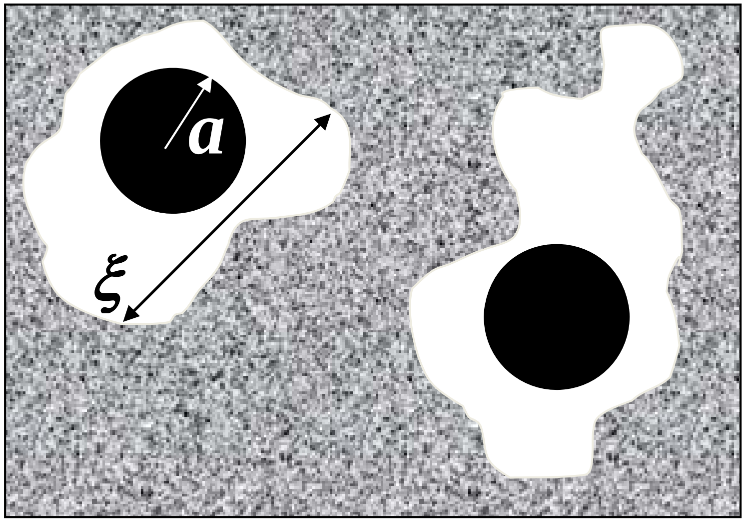
Dasgupta et al. PRE **65**, 051505 (2002)

Interpreting the MSD of tracers probing inhomogeneous materials can be tricky



But what about the boundary conditions???

Interpreting the MSD of tracers probing inhomogeneous materials can be tricky

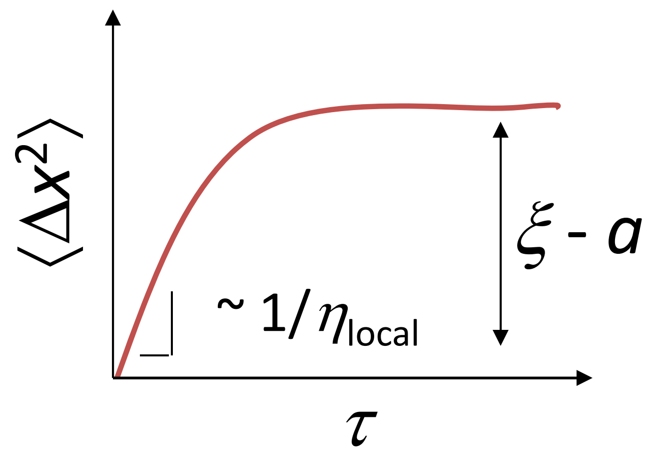


Particles are sensitive to both local viscoelasticity, and ***microstructure***.

if $a < \xi$, then particles move in small "pores"

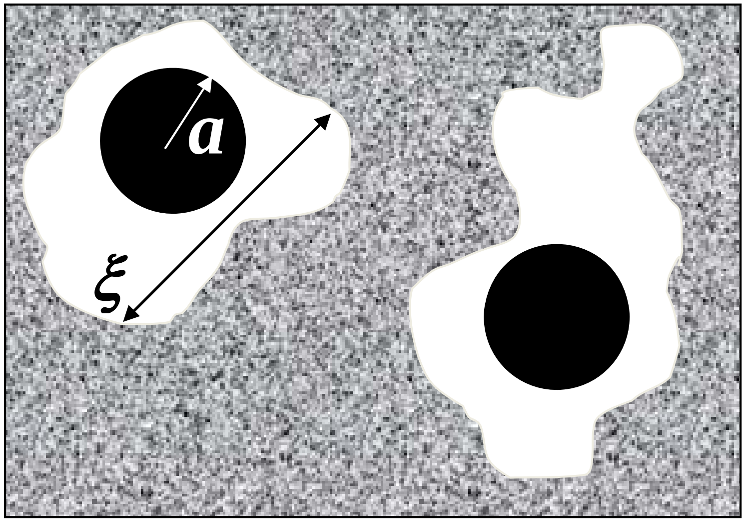
Measure "pore size", ξ:

$$\sqrt{\langle \Delta x^2(\tau) \rangle} + a = \xi$$



Use particles of different sizes to characterize constraint.

Interpreting the MSD of tracers probing inhomogeneous materials can be tricky



Particles are sensitive to both local viscoelasticity, and ***microstructure***.

if $a < \xi$, then particles move in small "pores"

Dirty Secret: Addition of the particles can actually introduce heterogeneity!

Depletion layers…

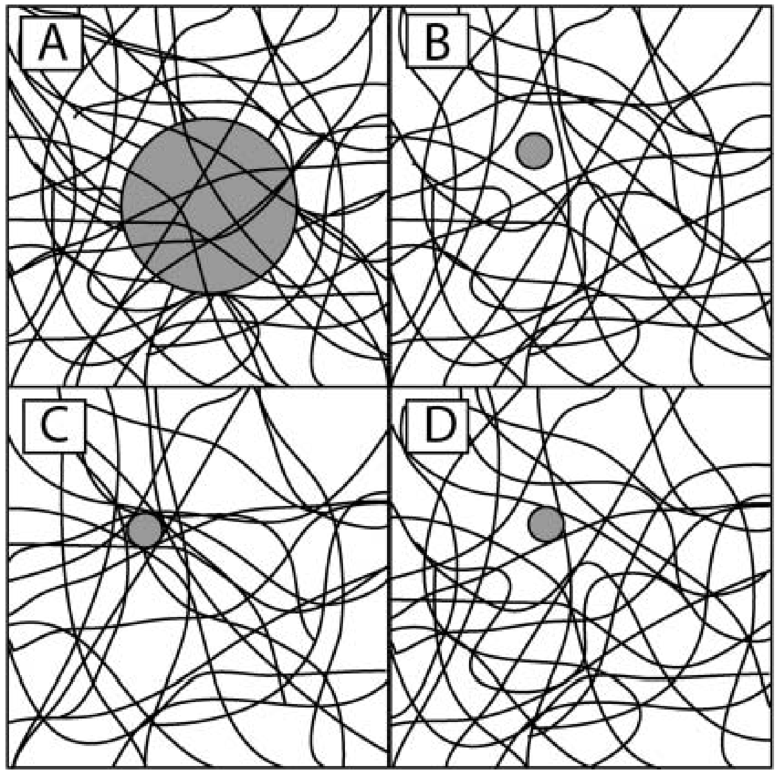
Polymer/particle adhesion effects…etc.

This can be a nightmare……

Particle-network interactions can also modify tracer motion

$a >> \xi$
Inert tracer
Measures $G^*(\omega)$

$a < \xi$
Very sticky tracer modifies local polymer concentration



$a < \xi$
Inert tracer
Measures η_{local}, ξ

$a < \xi$
Adherent tracer
Measures network fluctuations

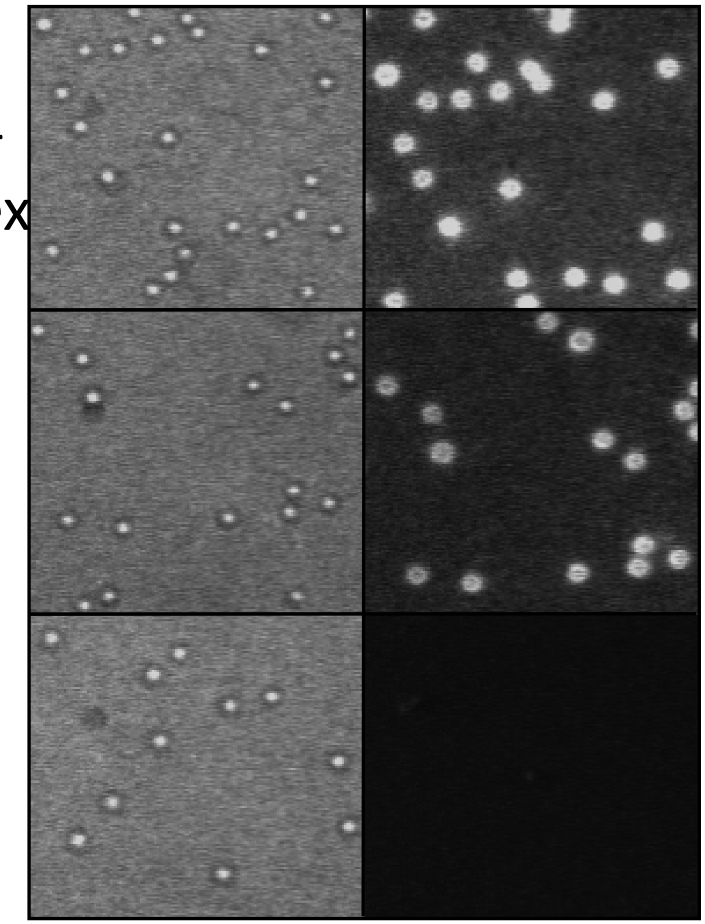
Valentine, Perlman, et al. *BJ* (2004).

Protein-binding capacity of colloids depends on surface chemistry

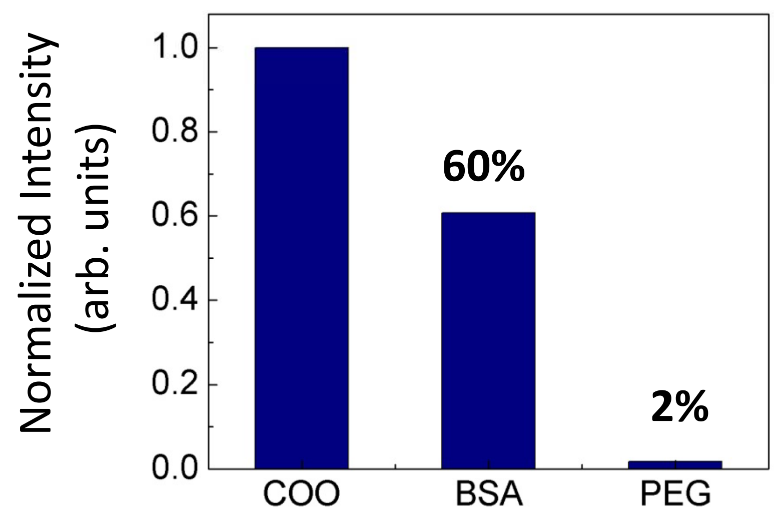
Untreated carboxylate-modified latex (CML)

Protein (BSA)-coated

Polymer (PEG)-coated

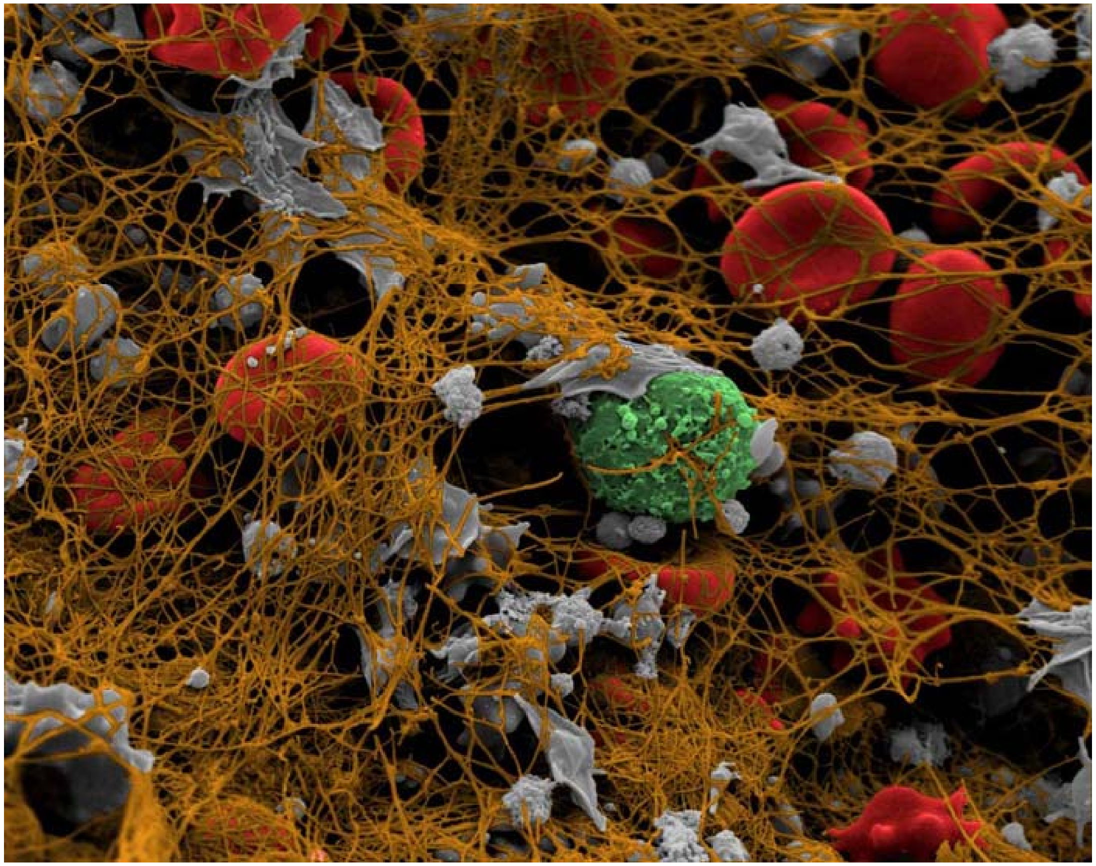


Particles are incubated with fluorescent-BSA



Valentine, Perlman, et al. *BJ* (2004).

Example: Fibrin, a blood clotting protein

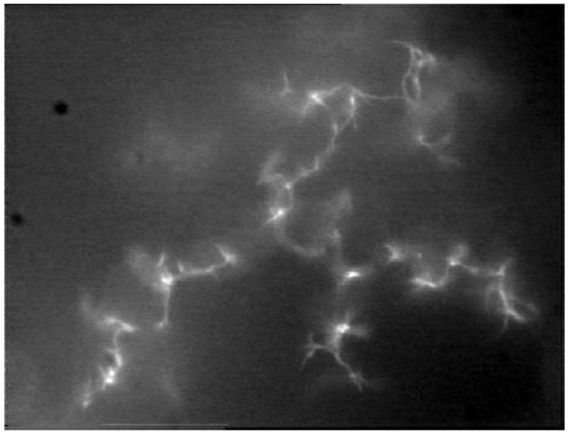


Colorized scanning electron micrograph of a coronary artery thrombus taken from a patient who had a heart attack.

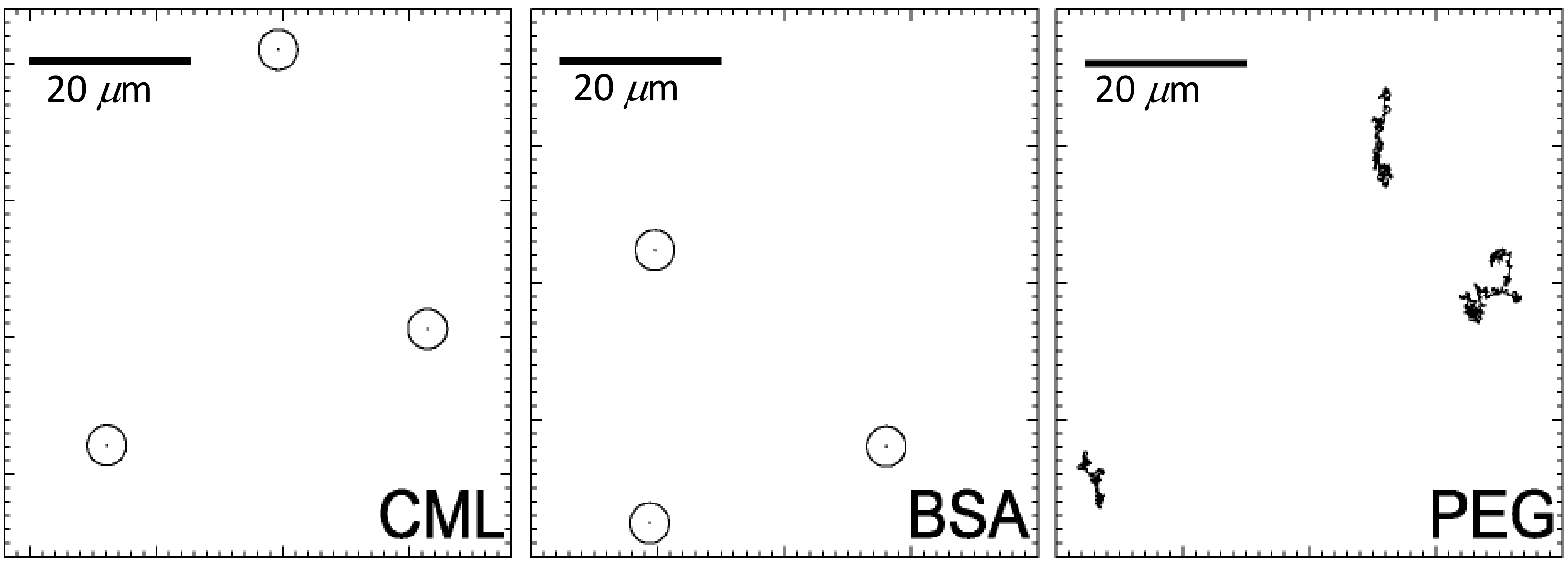
Fibrin fibers are brown,
Platelets are gray,
Red blood cells in red,
Leukocytes are green.

Credit: John Weisel, UPenn

Particle mobility depends on tracer-polymer interactions



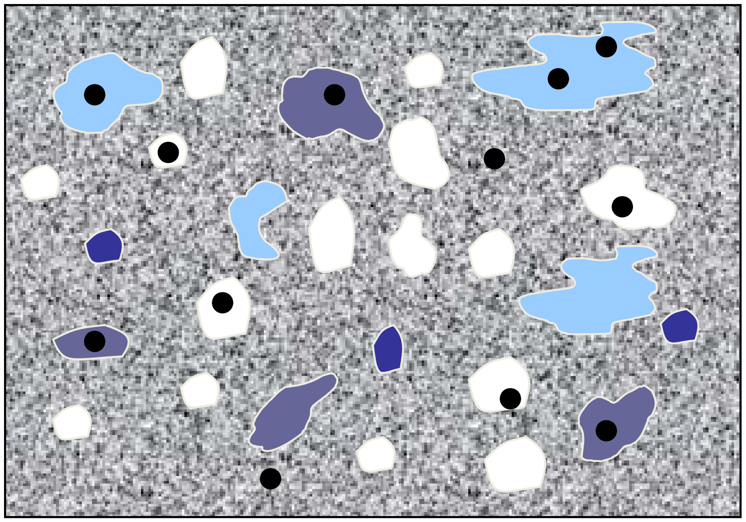
0.44 mg/mL **fibrin** network
ξ = 5-10 μm



a = 1 μm

Valentine, Perlman, et al. *BJ* (2004).

Multiple particle tracking measures variations in heterogeneous samples



- Simultaneously measure the thermal motions of dozens of particles.
- Possible to detect subtle differences in the mechanical microenvironments

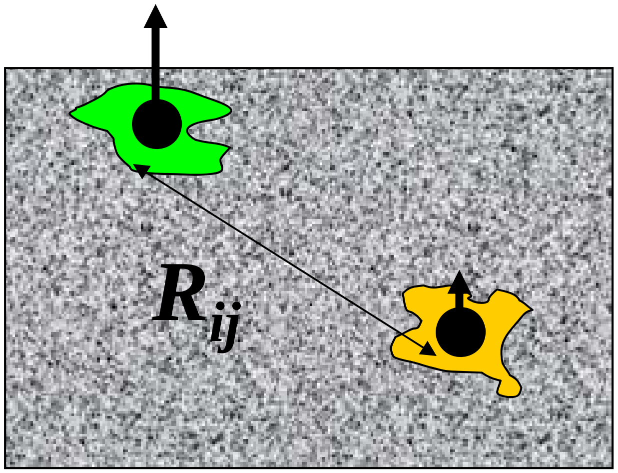
•In principle, can be combined with fluorescence readout to correlate structure-mechanics

Need a method that quantitatively compares neighboring particles

Valentine, Kaplan, et al. *PRE* (2001).

<u>2-particle Microrheology</u>: Measures long length-scale rheology for inhomogeneous materials

Basic Idea: Measure correlated motion of separated beads



$$\Delta r_\alpha(t,\tau) = r_\alpha(t+\tau) - r_\alpha(t)$$

α, β = coordinate axes
i, j = different particles

Calculate Tensor Product of Displacements:

$$D_{\alpha\beta}(r,\tau) = \left\langle \Delta r_\alpha^i(t,\tau)\Delta r_\beta^j(t,\tau)\delta(r - R^{ij}(t)) \right\rangle_{i\neq j,t}$$

Coarse-grained limit, $D_{rr} \sim 1/r$:

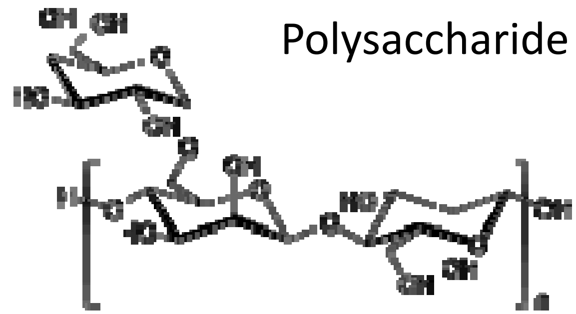
$$D_{rr}(r,s) = \frac{k_B T}{2\pi r s G(s)}$$

$$\langle \Delta r(\tau) \rangle_D = \frac{2r}{a} D_{rr}(r,\tau)$$

Crocker, Valentine, et al. *PRL* (2000).

Guar: Food thickener, very heterogeneous

Guar
Plant

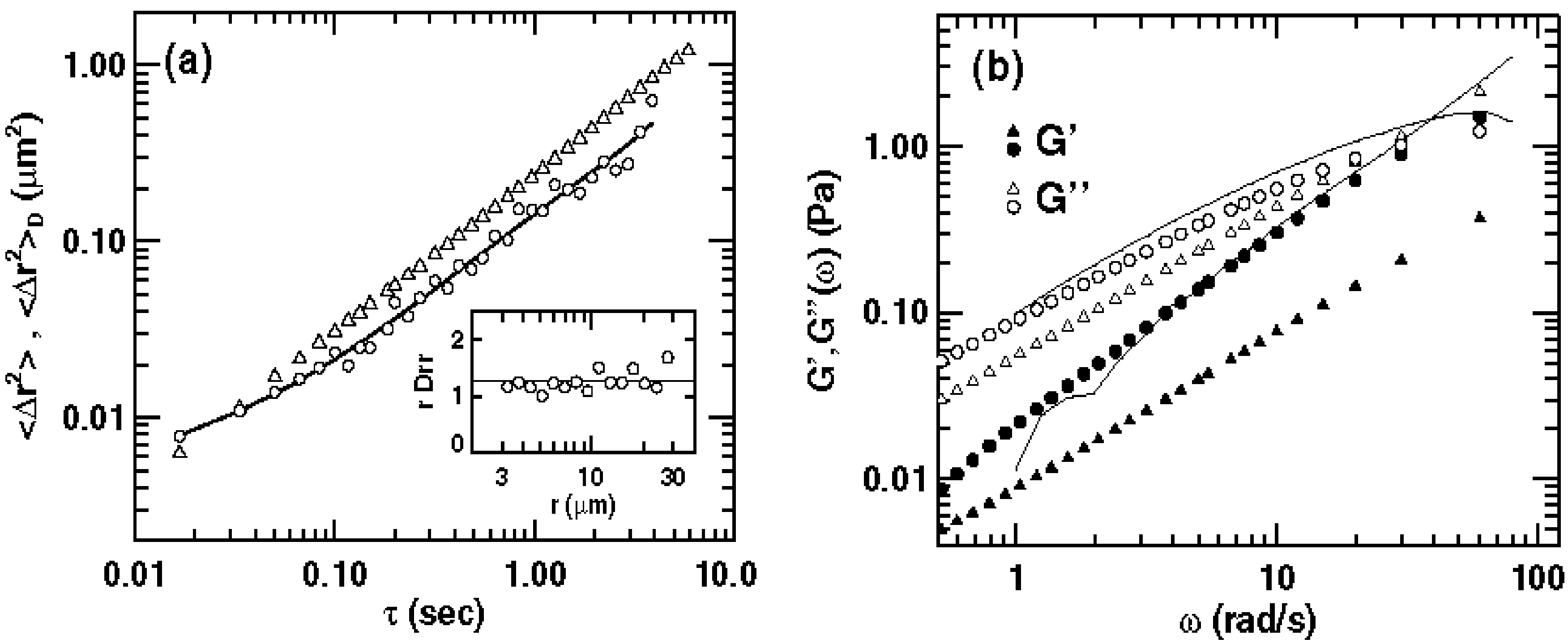


Polysaccharide

Beans

Thickener
“Mouth Feel”

Example: Heterogeneous Guar Solution

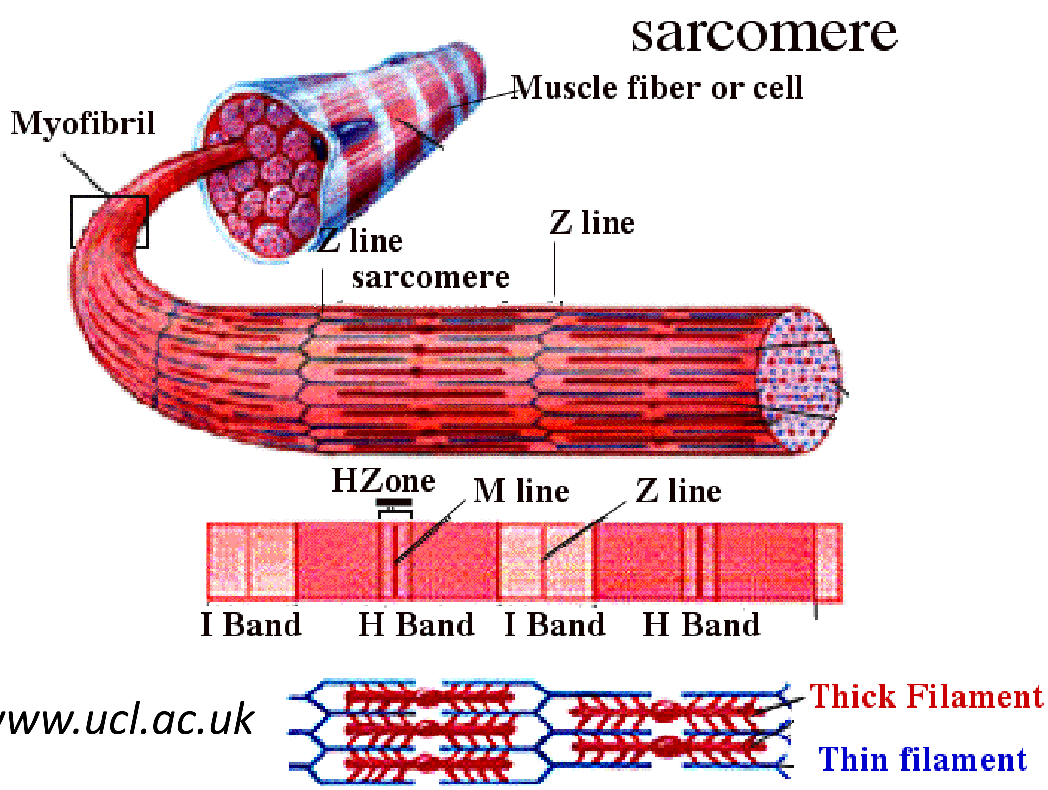


(△) one-particle MSD

(-○-) two-particle MSD

Crocker, Valentine, et al. *PRL* (2000).

Example: Filamentous Actin

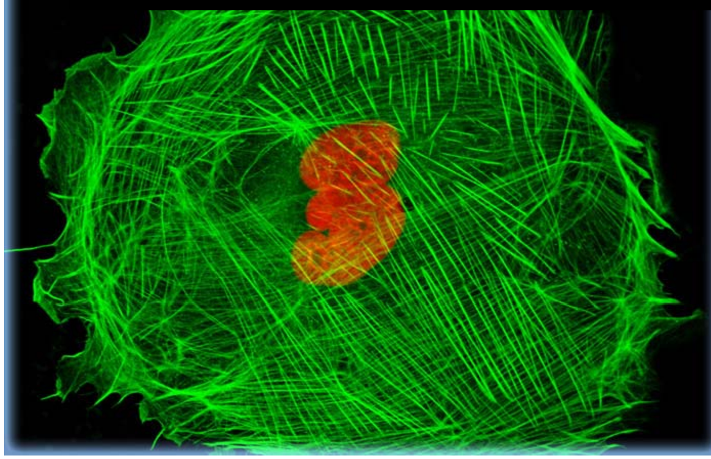


www.ucl.ac.uk

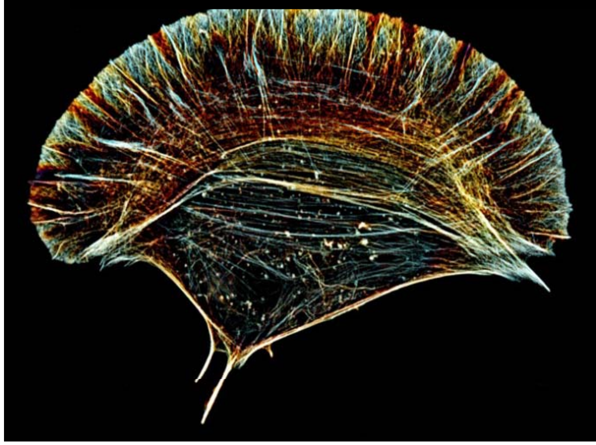
Yum!

Actin
Myosin

opposum kidney epithelial cells

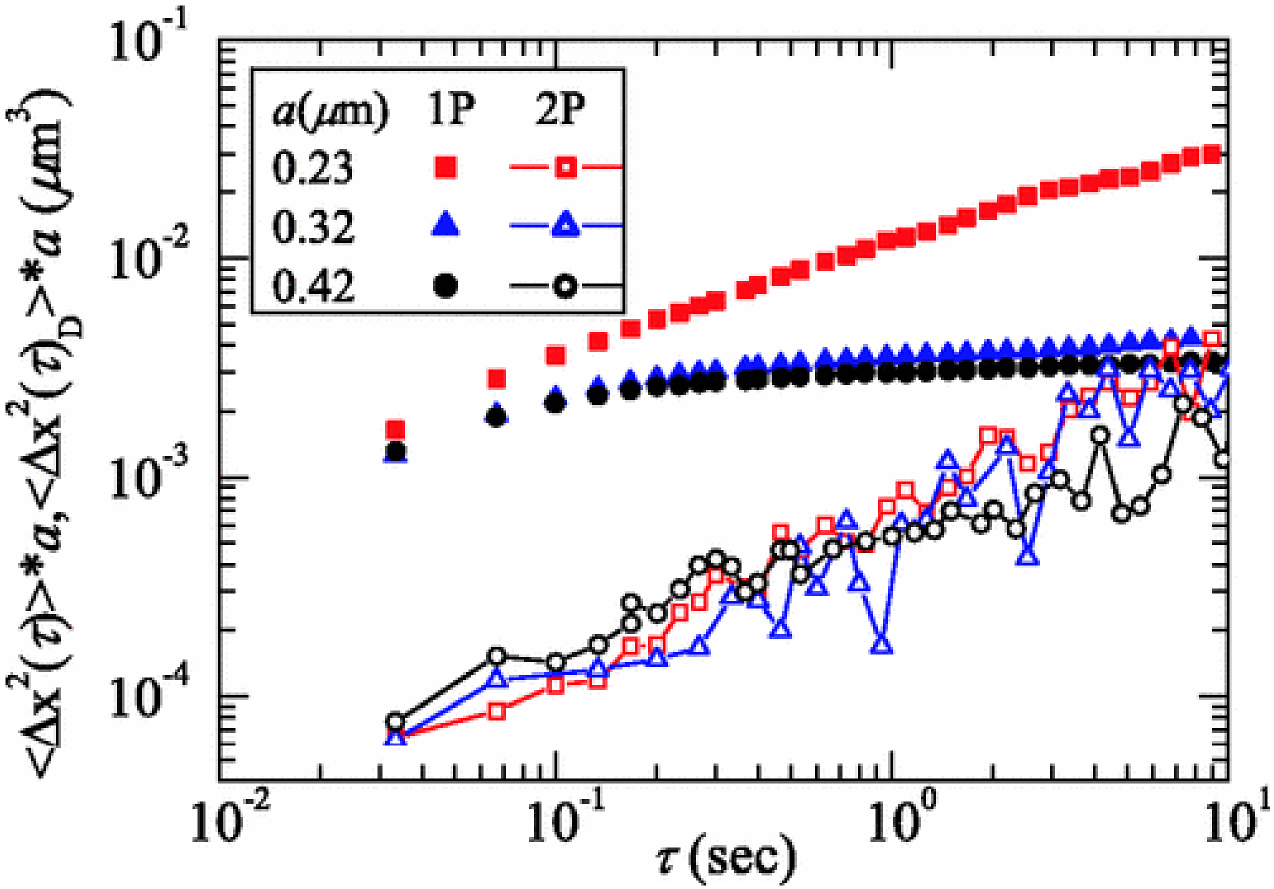


Mouse embryonic fibroblast



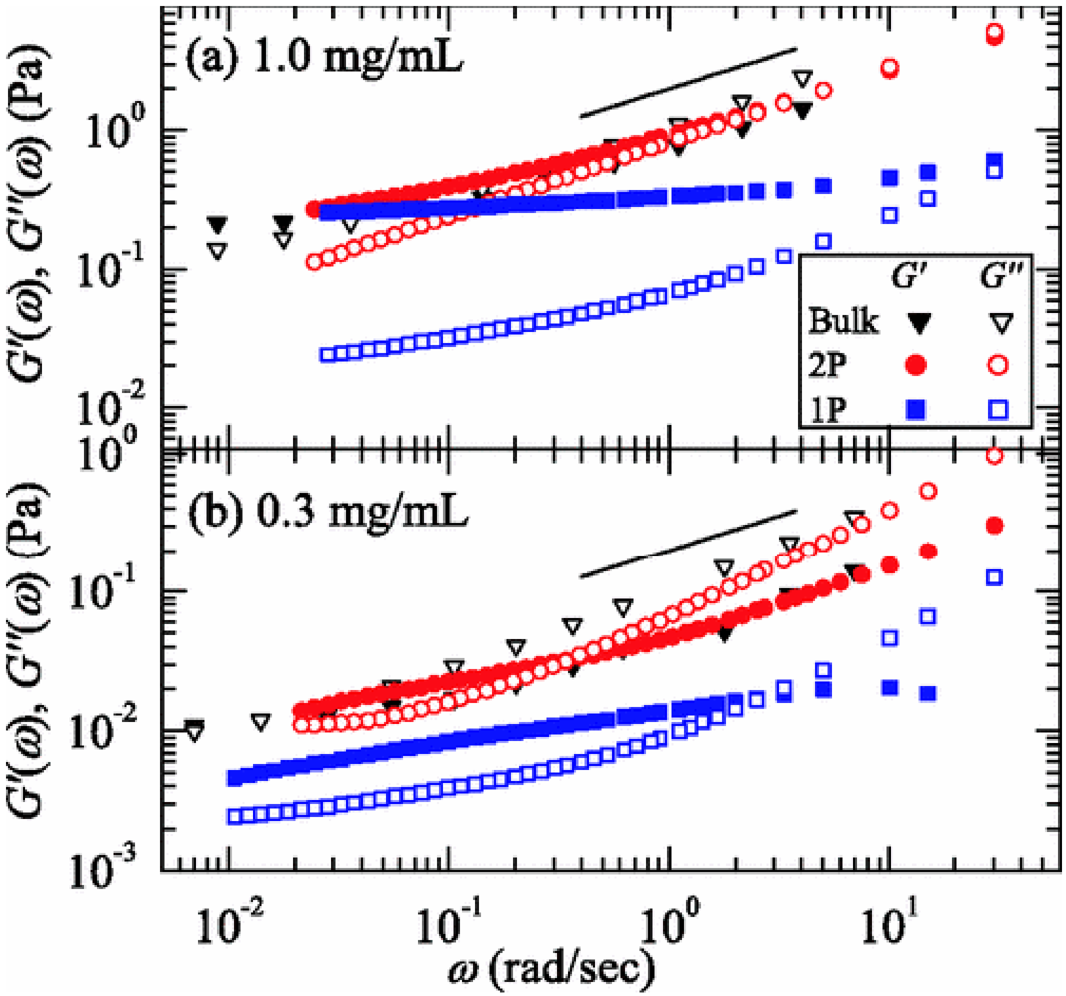
Olympus. com

Example: Filamentous Actin



M. L. Gardel, M. T. Valentine, J. C. Crocker, A. R. Bausch, and D. A. Weitz; Phys. Rev. Lett. 91, 158302 (2003)

Example: Filamentous Actin

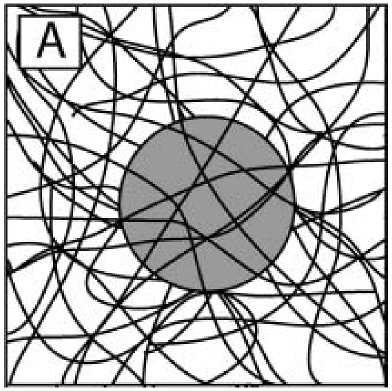


M. L. Gardel, M. T. Valentine, J. C. Crocker, A. R. Bausch, and D. A. Weitz; Phys. Rev. Lett. 91, 158302 (2003)

Bottom Line….

• Measuring rheology of soft materials is difficult, biopolymers particularly so

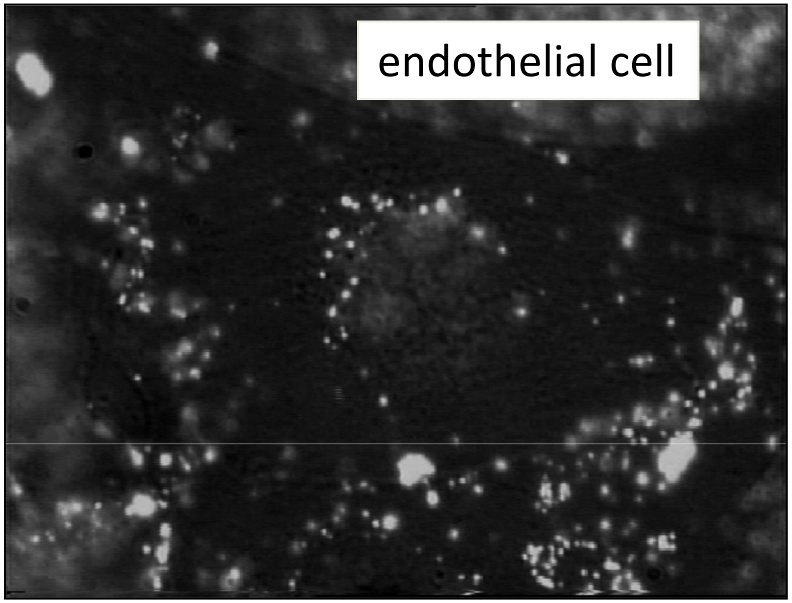
• Microscale techniques are an excellent option, allowing measurement of heterogeneous materials and direct correlation between structure and mechanics



• **But be careful in interpretation**

- Particles can disturb local network structure
- Surface chemistry matters
    - *Slip can also be a problem in rheometers*
- Structural confinement and elastic deformation give similar MSDs
- Statistical noise can be high

In motile, force-generating systems, interpretation is more difficult



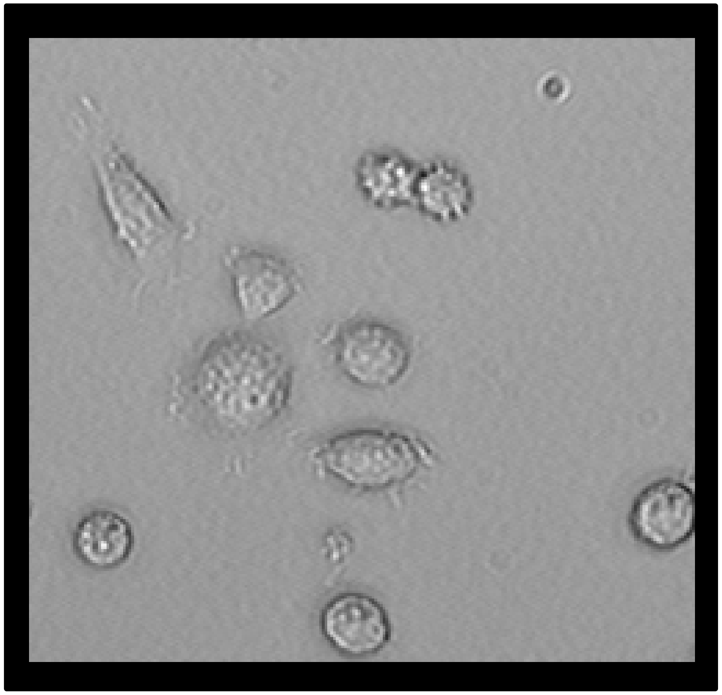
Systems are not only heterogeneous, but out-of-equilibrium.

New energy sources!

$$Energy \neq k_B T$$

$$\eta(\tau) \neq \frac{k_B T \tau}{3\pi a \langle \Delta x^2(\tau) \rangle}$$

In motile, force-generating systems, interpretation is more difficult



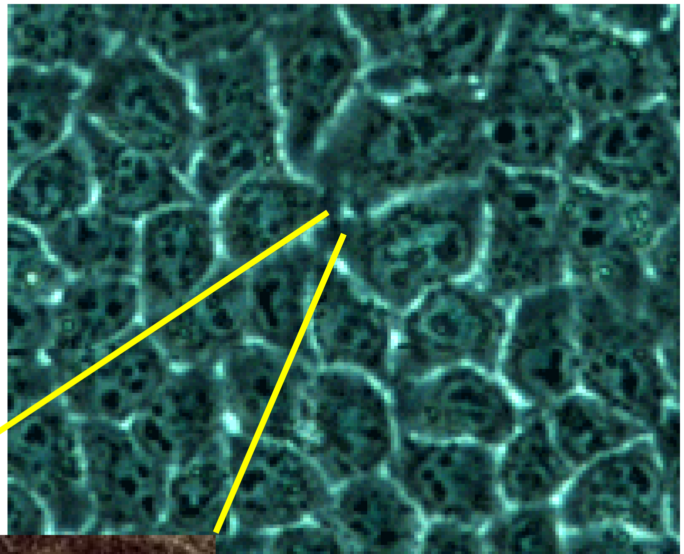
Systems are not only heterogeneous, but out-of-equilibrium.

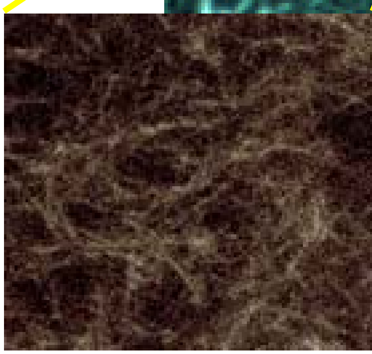
New energy sources!

$$Energy \neq k_B T$$

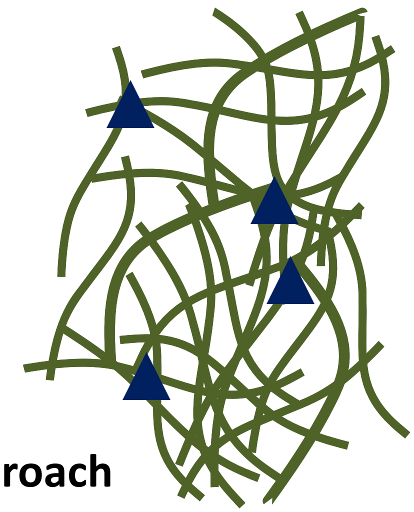
$$\eta(\tau) \neq \frac{k_B T \tau}{3\pi a \langle \Delta x^2(\tau) \rangle}$$

Cytoskeleton: Internal biopolymer network that regulates shape, stiffness





Dense polymer networks

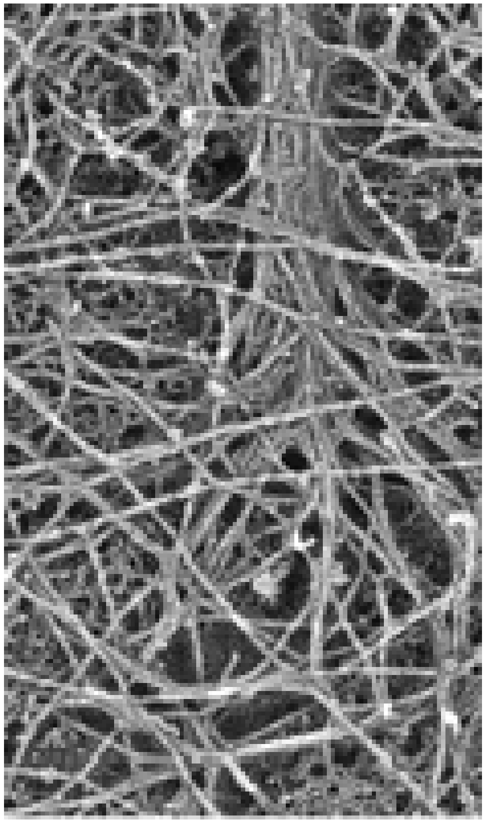


Reductionist Approach

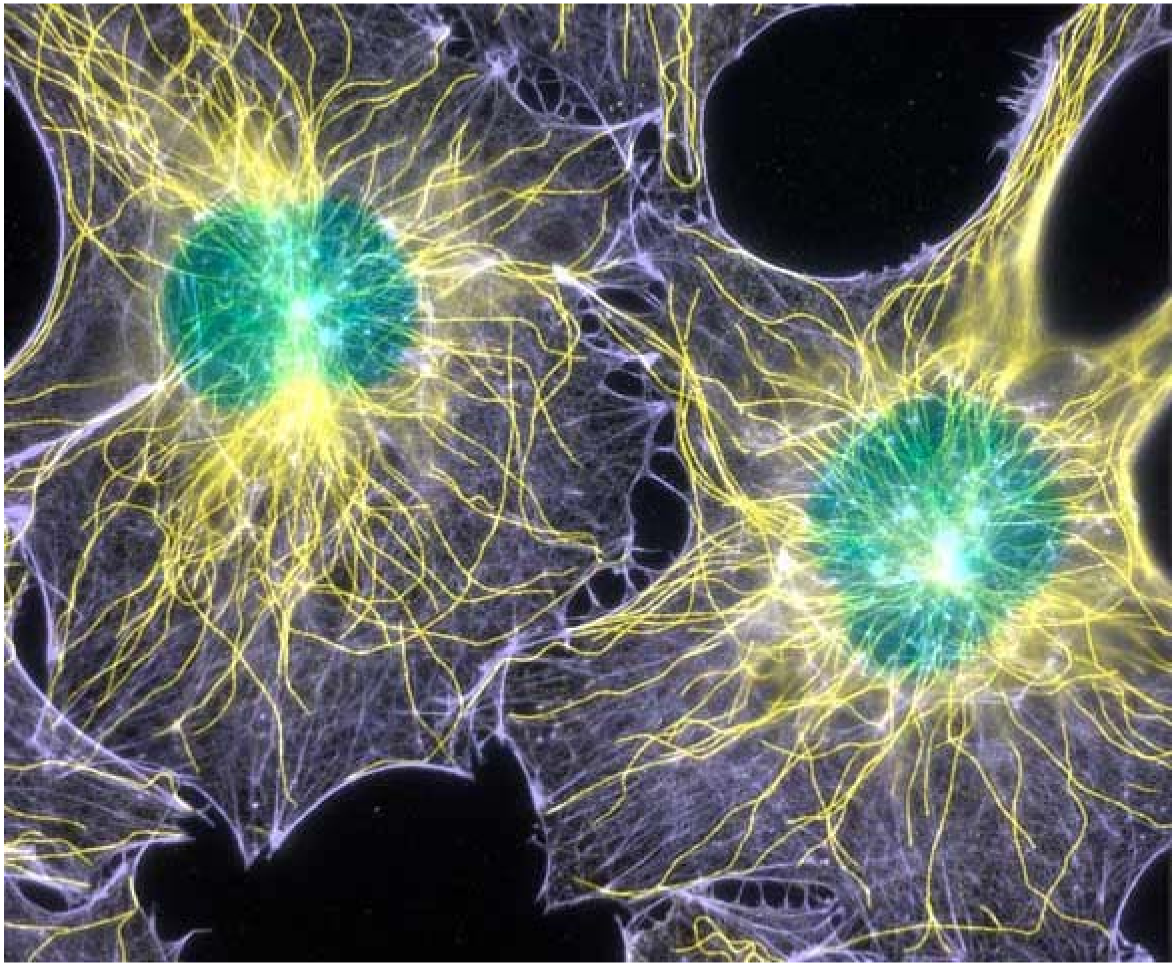
Primary Functions:

- Organizes cells
- Provides structural support & strength
- Serves as scaffold for assembly of other proteins
- Senses changes in chemical and mechanical environment
- Responds by growing or shrinking
- Generates/transmits force

Cytoskeletal Structures



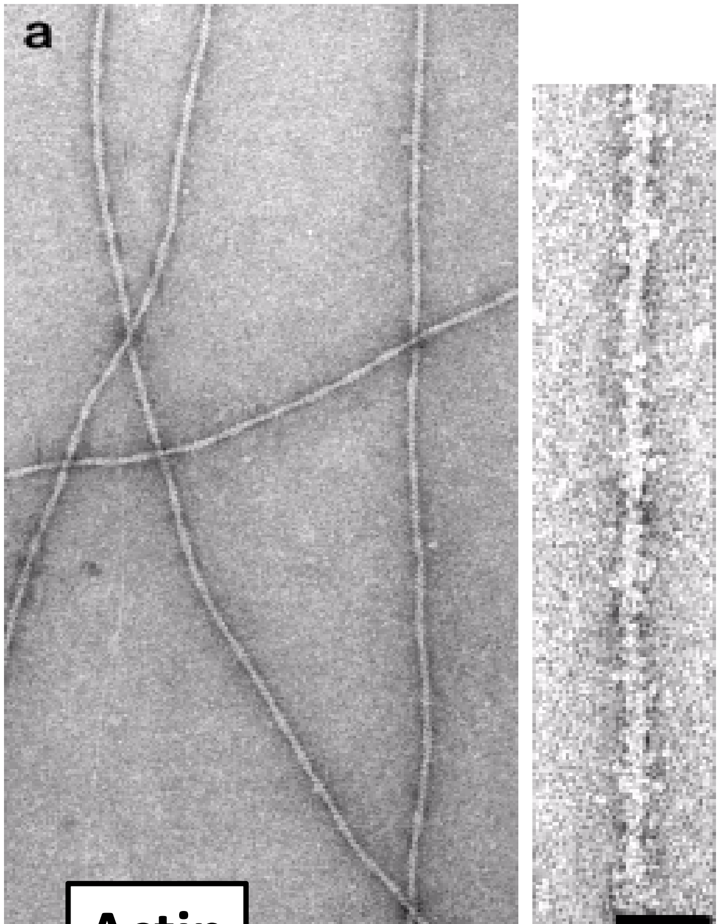
Electron micrograph
Svitkina *et al.* Cell (2003)



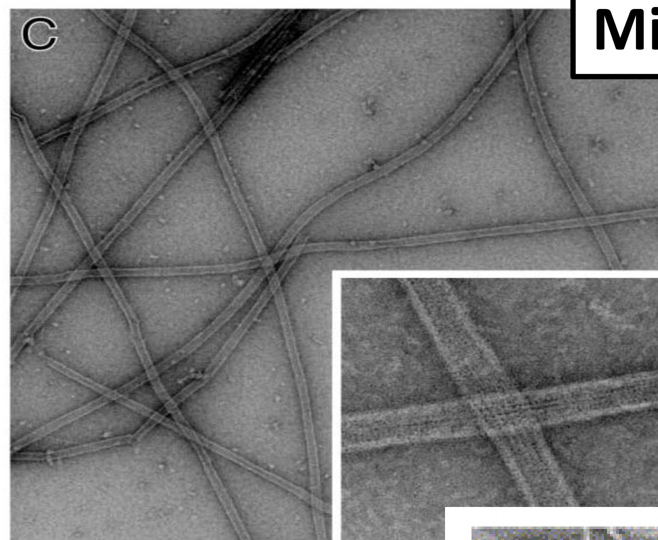
Not simple entangled networks!

Fluorescence microscopy
Image acquired by Torsten Wittmann; http://dir.nhlbi.nih.gov/labs/lctm/

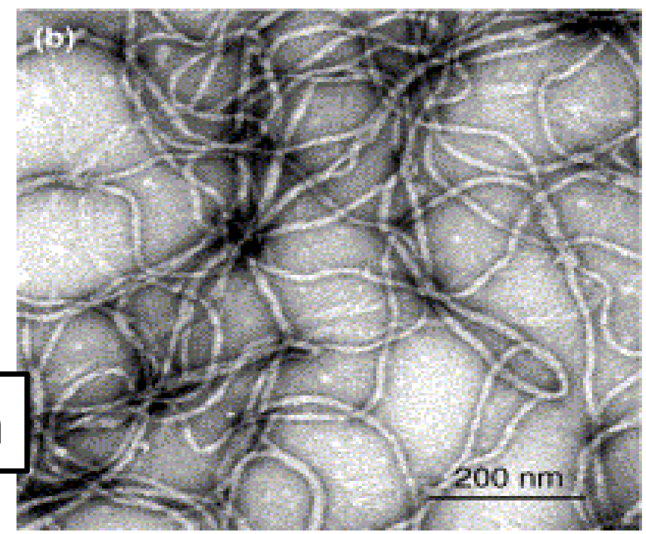
Filaments were originally identified by electron microscopy



Actin



Microtubules



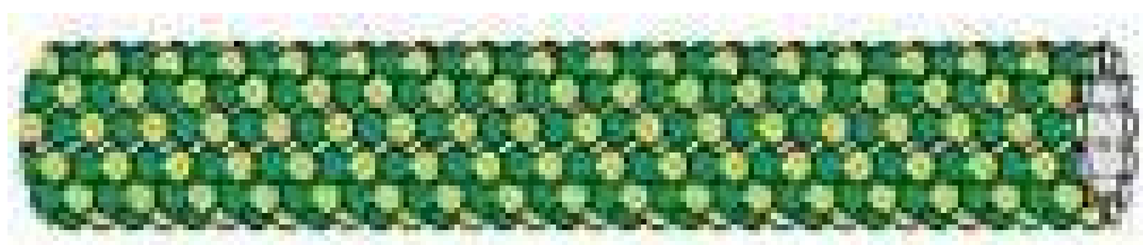
IF: Keratin

Eukaryotic cells contain 3 major classes of cytoskeletal filaments

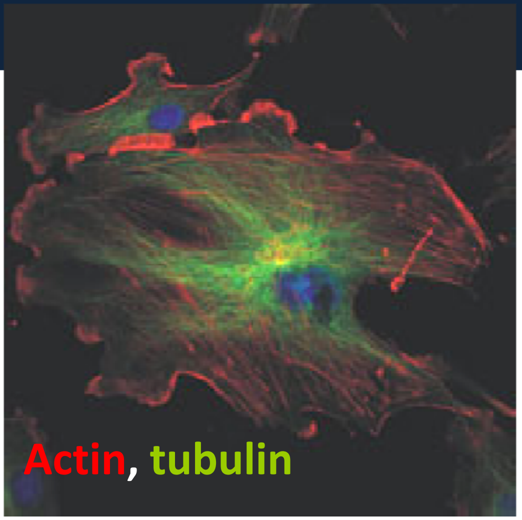
- Actin filaments (*d* ~ 6 nm)
  - Also known an microfilaments
  - Cable-like

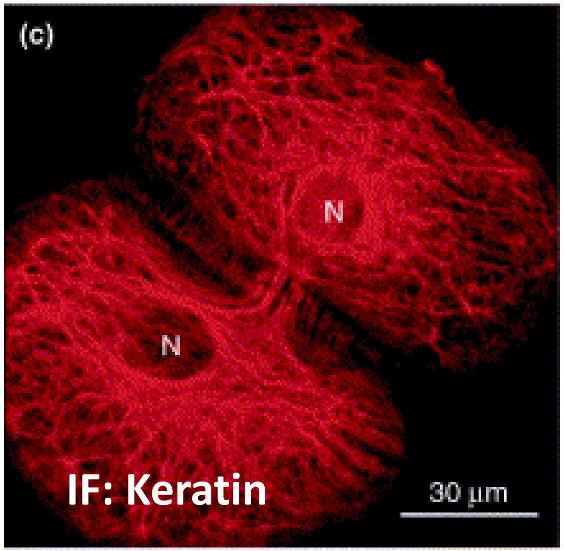
- Intermediate Filaments (*d* ~ 10 nm)
  - Rope-like

- Microtubules (d_o ~ 25 nm; d_i ~ 18 nm)
  - Pipe-like



Cytoskeleton: Function





Actin

Motility, mechanical strength,
Localized to edge, cytokinesis

Intermediate Filaments

Cell-to-cell contacts

Microtubules

Transport, Organization, Division

Changes in polymerization state dramatically affect mechanical response, but have little biochemical signature

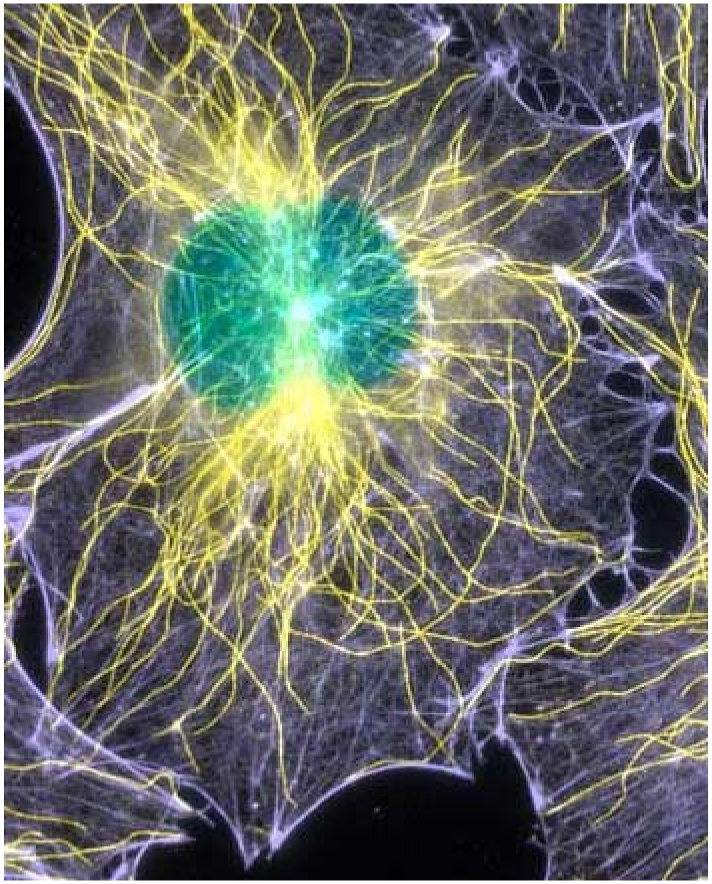
Implications to rheology

- Filaments are multistranded
  - Grow/shrink mainly from ends
  - Long
  - Stiff

- **Many binding partners that crosslink, sever, nucleate**

- Also motor proteins that slide, generate force

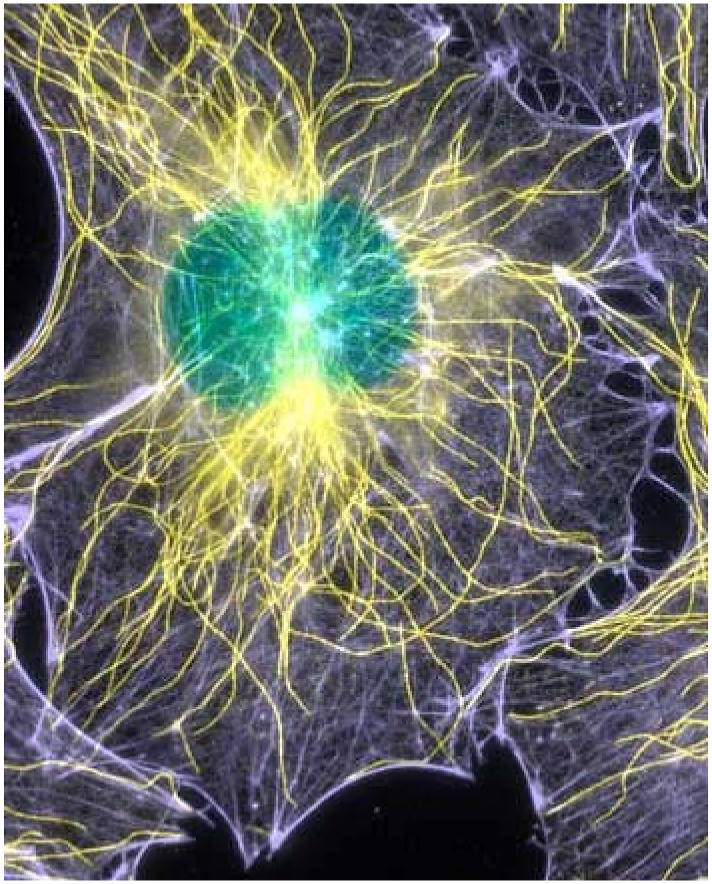
- Dynamic changes in architecture



http://dir.nhlbi.nih.gov/labs/lctm/

Highlights of cytoskeletal mechanics

1. Cytoskeletal polymers are very **stiff**
2. Network mechanics is often dominated by **crosslinker** properties
3. **Enzymatic activity** can be really important



http://dir.nhlbi.nih.gov/labs/lctm/

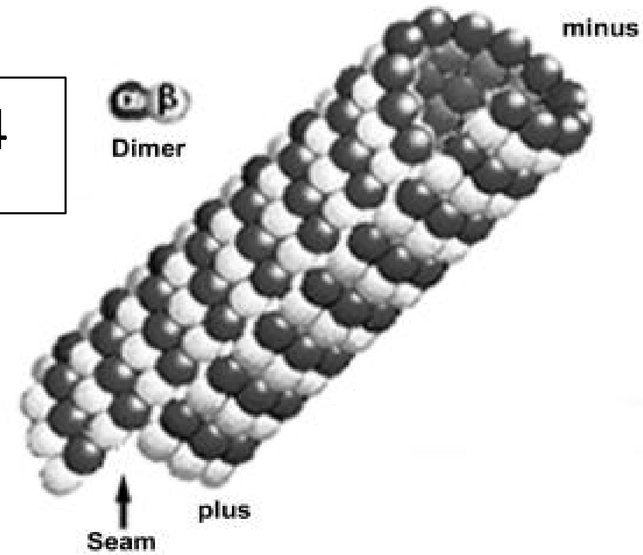
Microtubules are extraordinarily stiff, making them excellent model systems for rigid rods

Taxol-stabilized to suppress dynamics

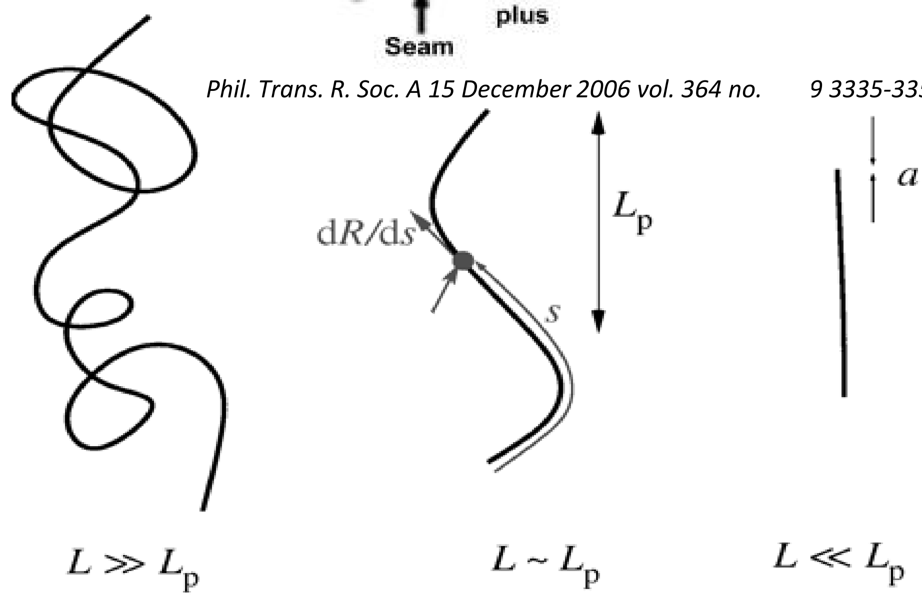
$L \sim 20\ \mu\text{m};\ L_p \sim 1\ \text{mm}$

Valdman, Atzberger, Yu, Kuei, Valentine. Biophys. J., 2012
Valdman, Lopez, Valentine , Atzberger. Soft Matter 2013
Hawkins, Ross et al. J. Biomechanics 2010
Hawkins, Ross et al. Biophys J. 2013
Gittes et al, JCB 1993

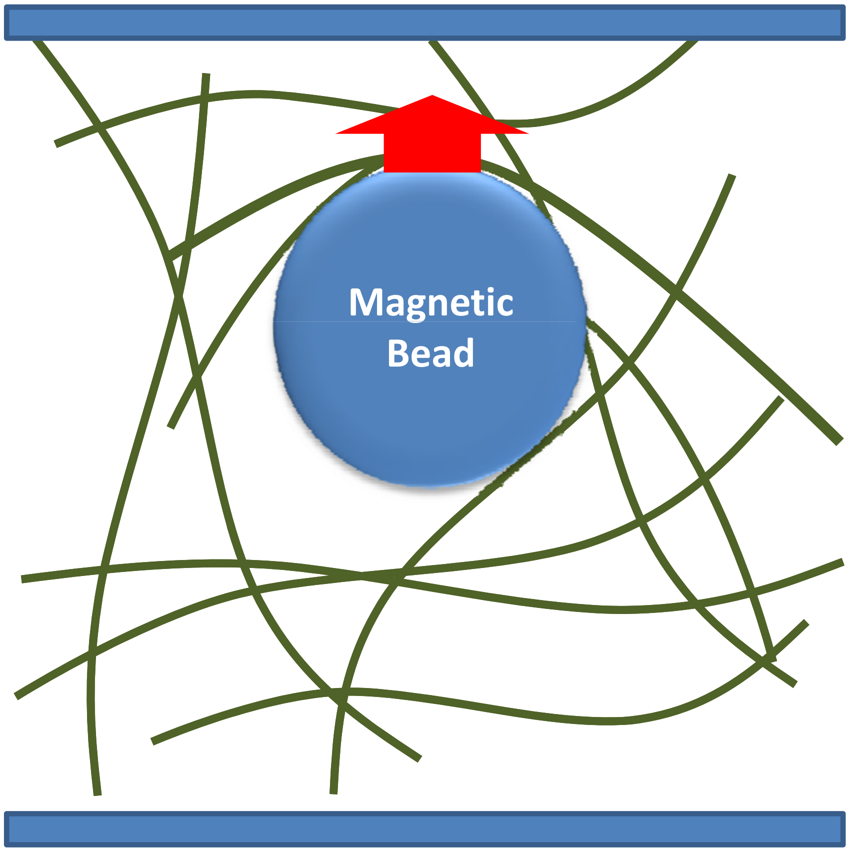
$$L_{\mathrm{P}} \sim r^{4}$$

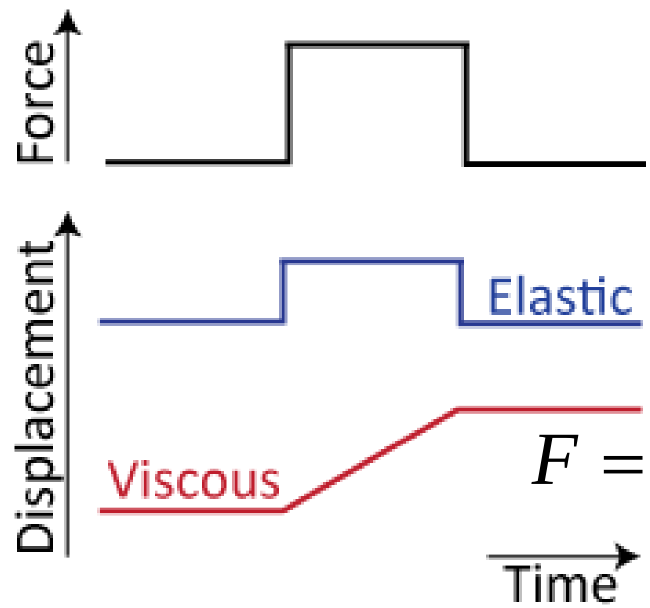


Phil. Trans. R. Soc. A 15 December 2006 vol. 364 no. 9 3335-3355



Microscale creep measurements with magnetic tweezers





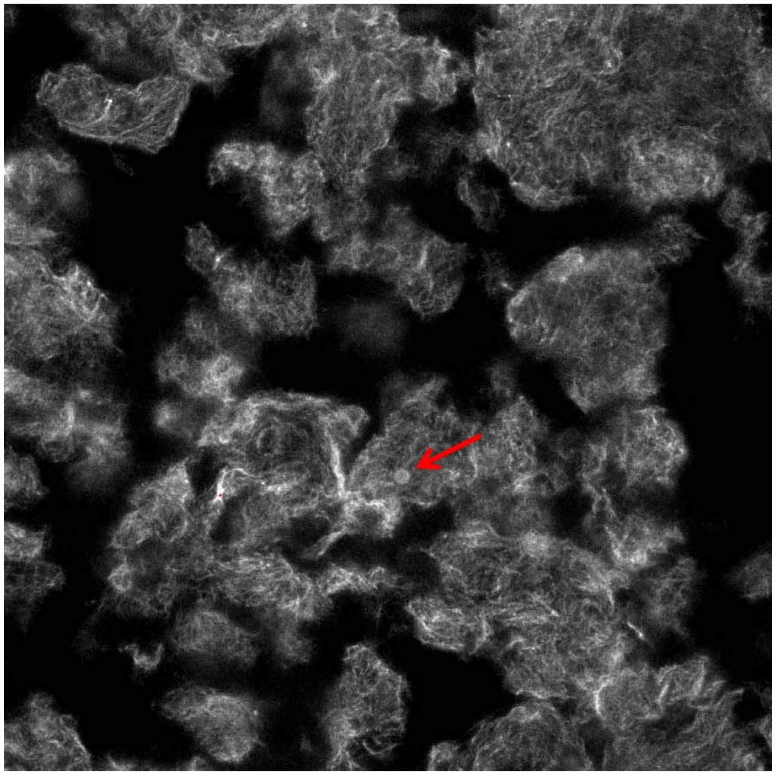
$$F = \kappa x$$

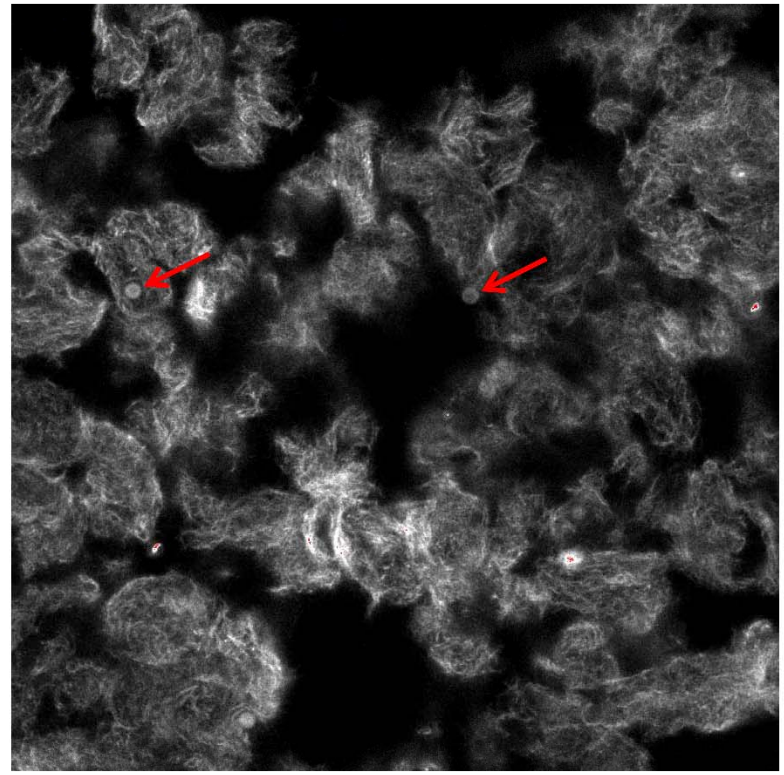
$$F = 6\pi\eta a \frac{dx}{dt}$$

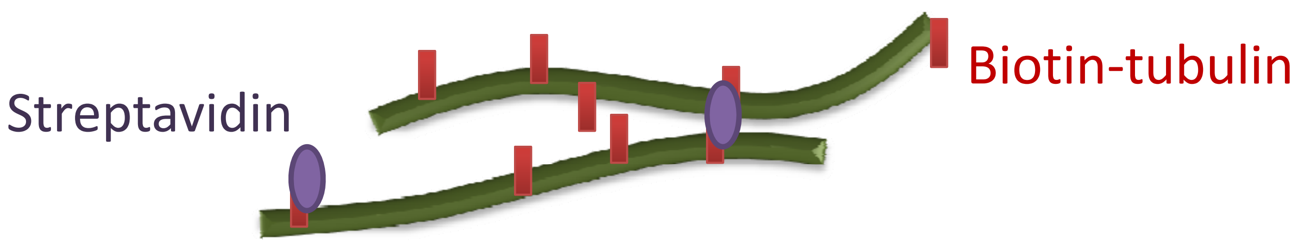
Microtubule Network Rheology
Yang, Lin, Kaytanli, Saleh and MTV, *Soft Matter* (2012)
Yang, Bai, Levine, Klug and MTV, *Soft Matter* (2013)
Yang and MTV, *Methods in Cell Biology* (2013)

Development of magnetic tweezers for microrheology
Lin and MTV, Review of Scientific Instruments (2012)
Lin and MTV, Applied Physics Letters (2012)
Zacchia and MTV, Review of Scientific Instruments (2015)

Strongly crosslinked networks, with biotin-streptavidin bonding



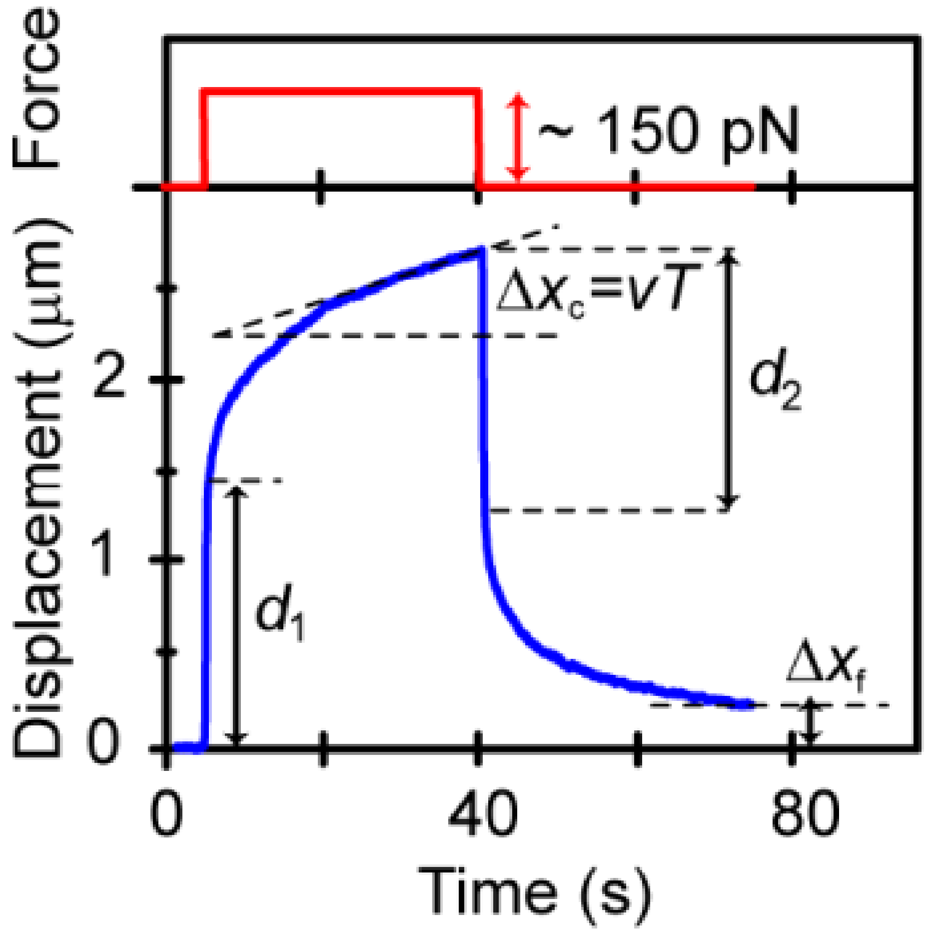




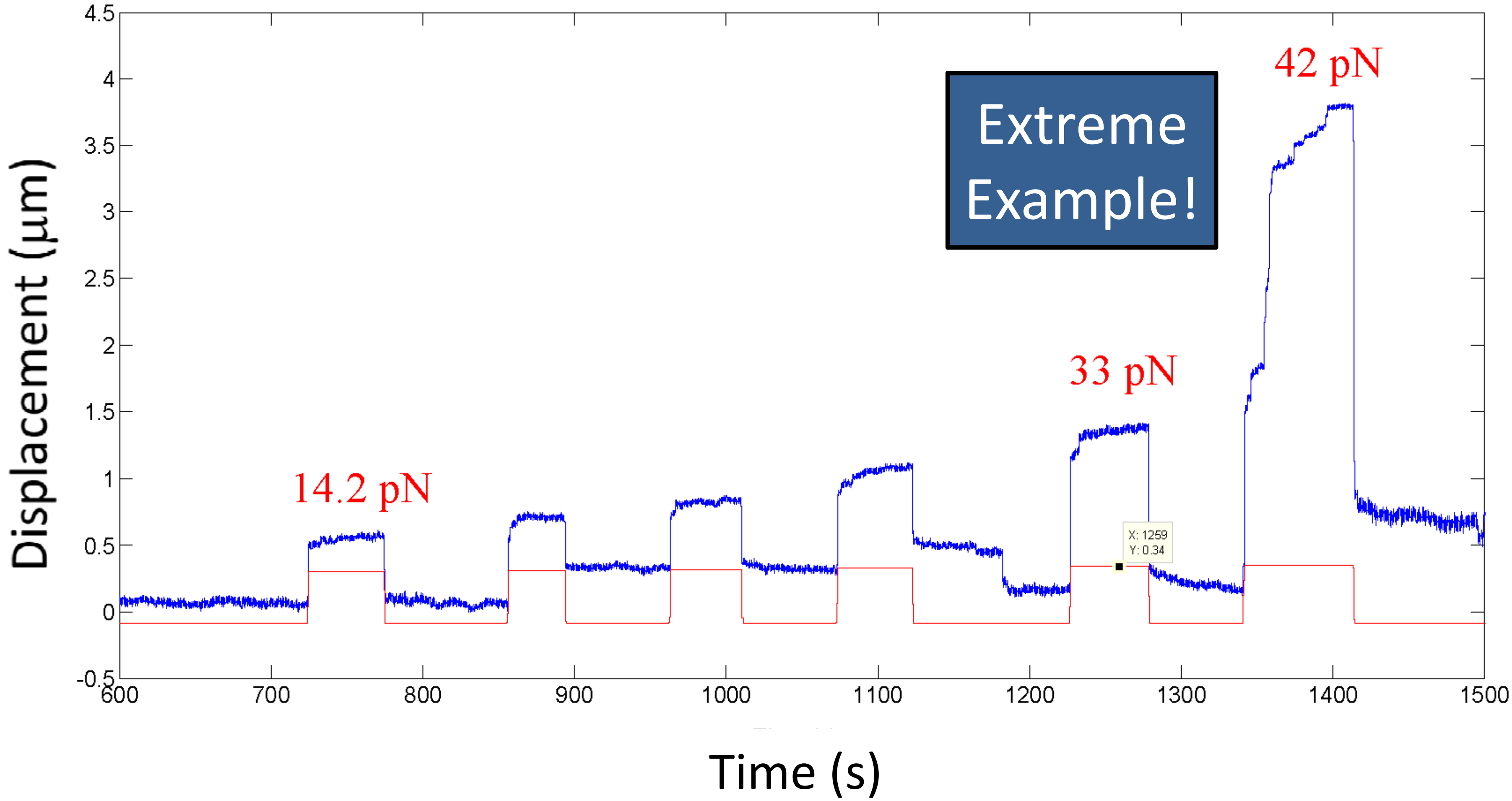
25 μM tubulin

Streptavidin:biotin **1:2**; 1:4 tubulin dimers is biotinylated (***R*** **= 25%**)

Typical response of bead to step force

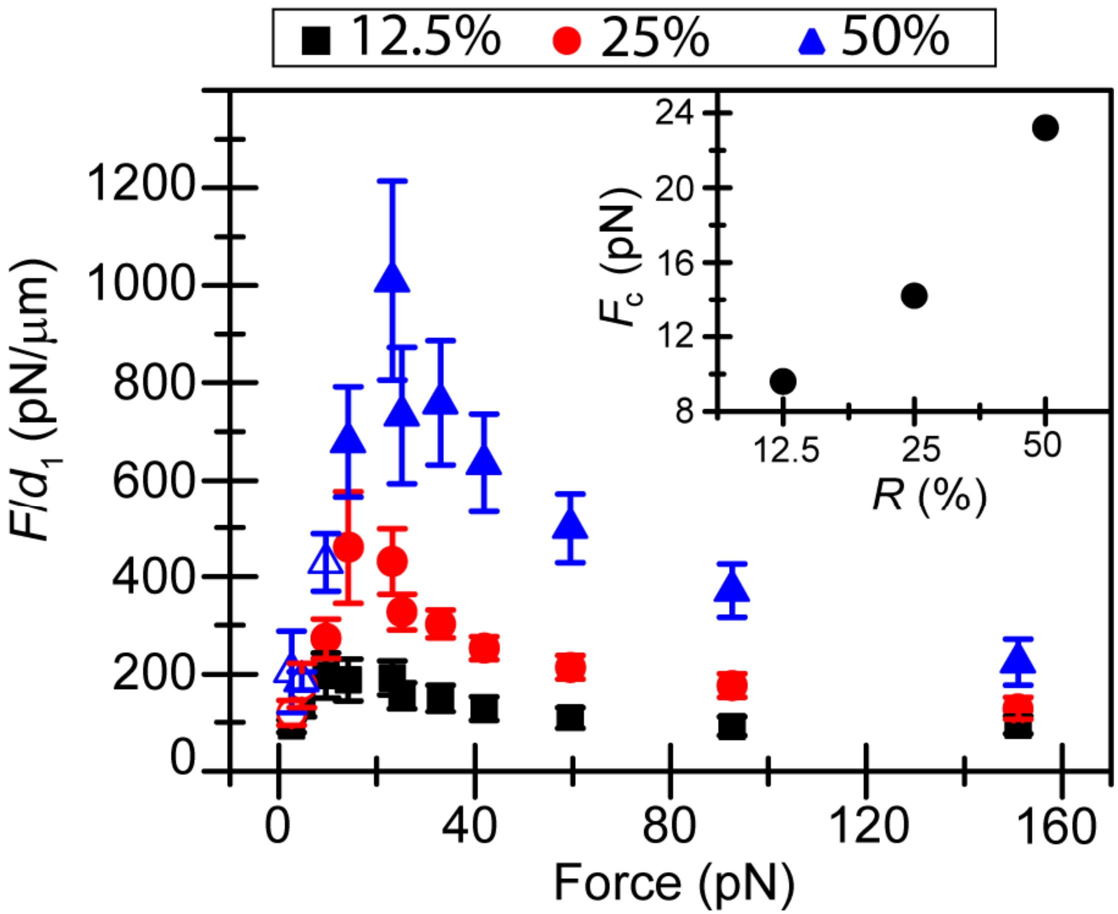


Frequent interactions with microstructure

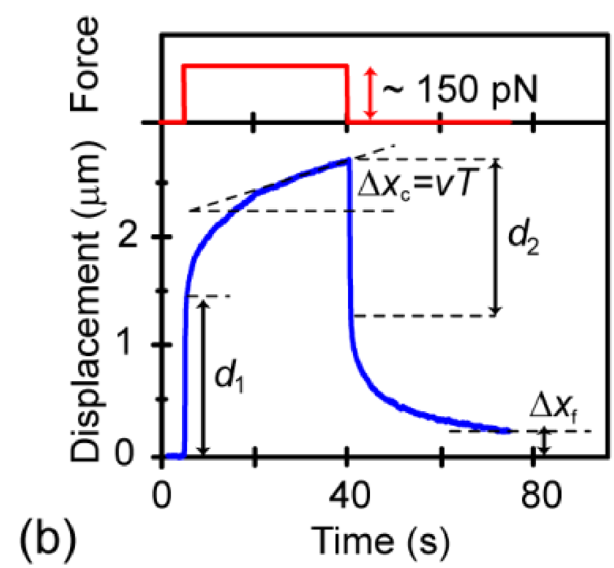


Vaca, Shlomovitz, Yang, Valentine, Levine, Soft Matter (2015)

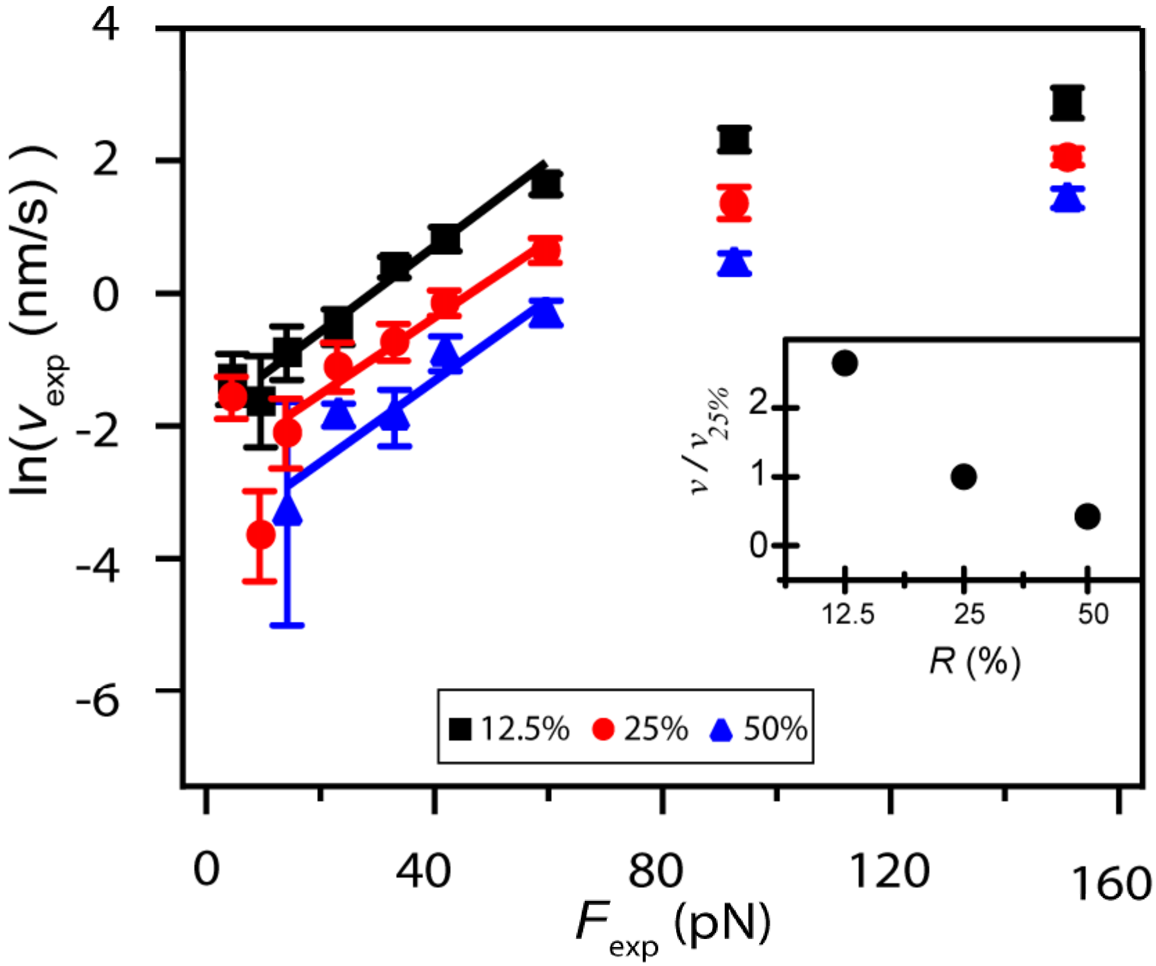
Elastic response is nonlinear with force



- *Stiffening at small forces*
- *Peak force and peak stiffness increase with crosslinking ratio R*
- *Softening at large forces*



Bead velocity increases exponentially with force for $F > F_c$

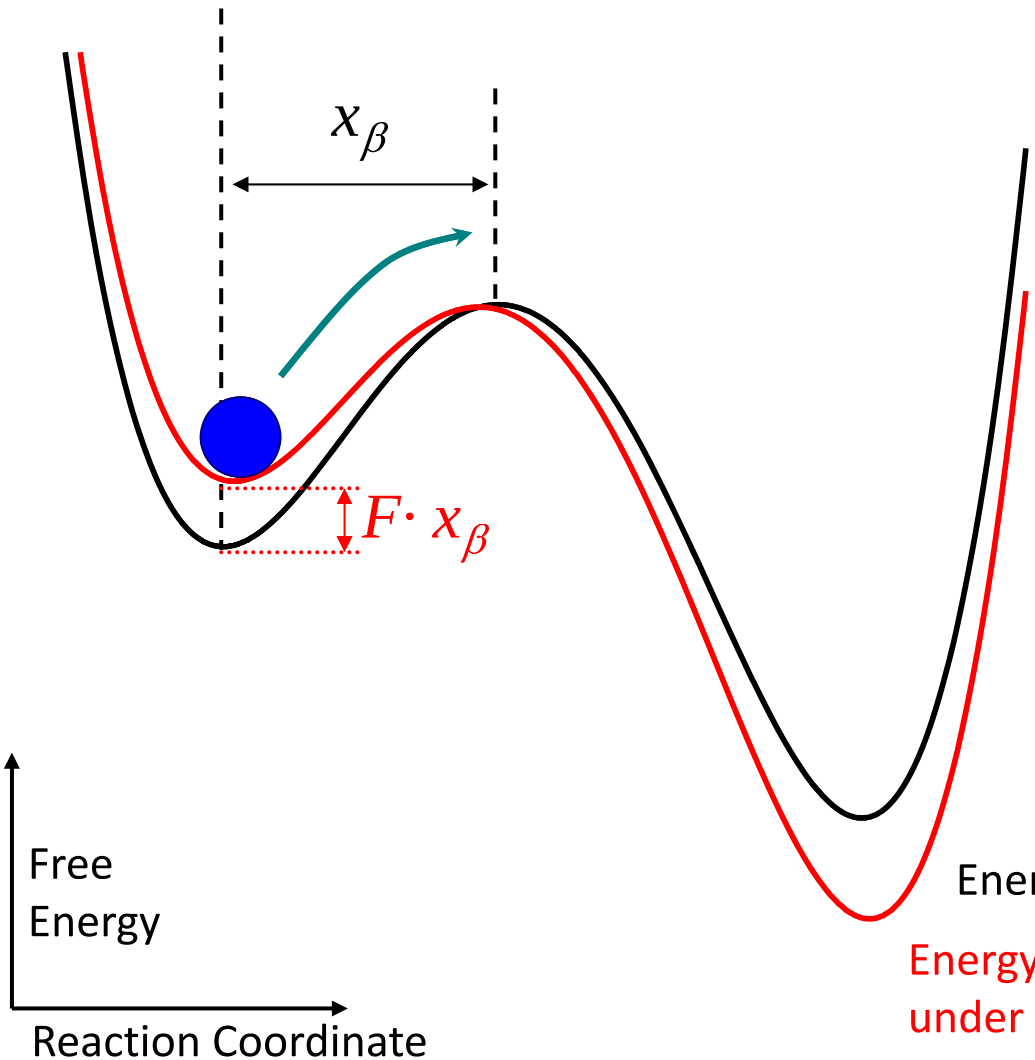


Consistent with a bond breakage mechanism

$$k_{off} = k_0 \exp(F x_\beta / k_B T)$$

$F < 65$ pN, $x_\beta \sim 0.25$ nm
$F > 85$ pN, $x_\beta \sim 0.05$ nm

Force tilts the energy landscape of mechanical transitions



Bell Model

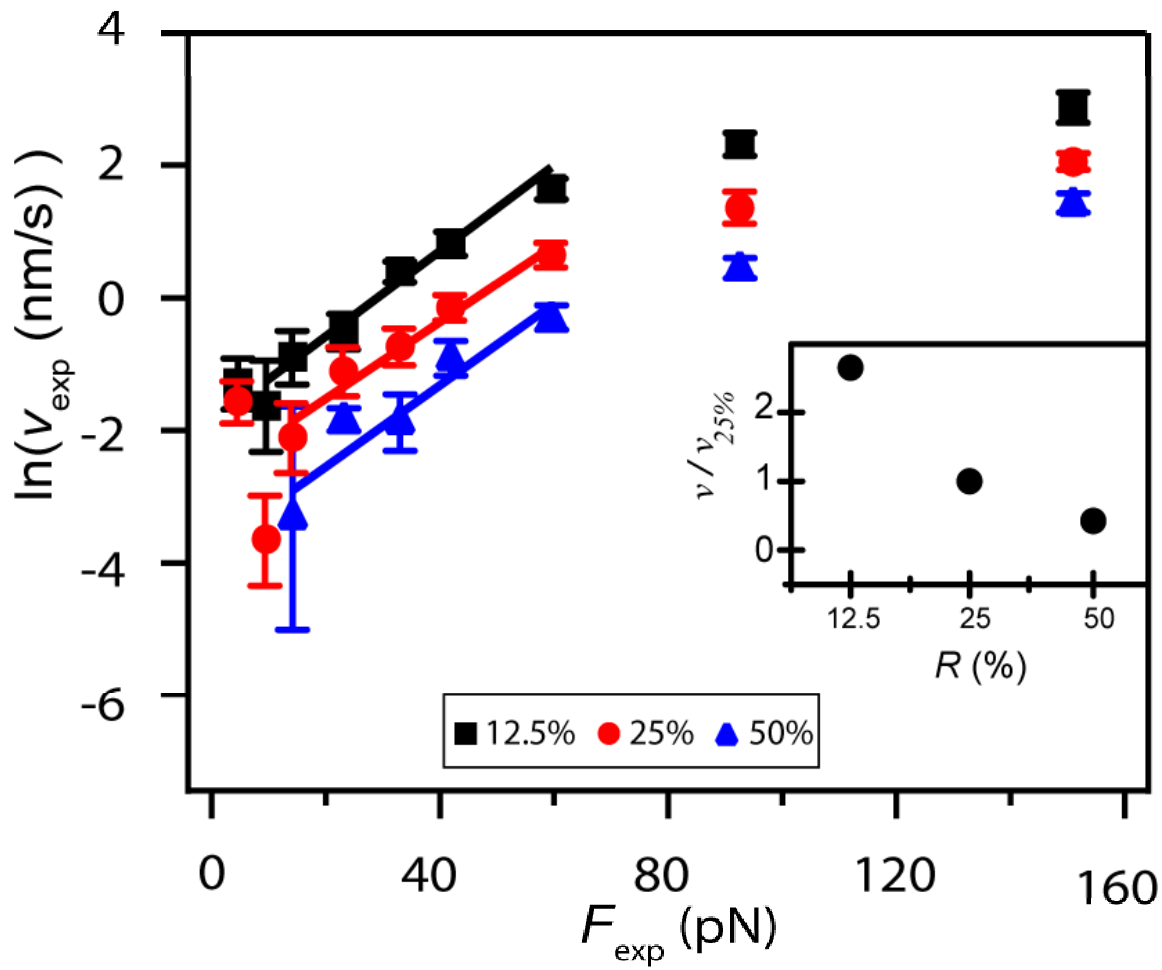
Transition rate:

$$k \propto \exp\left(Fx_\beta / k_B T\right)$$

GI Bell *Science* 1978
Dudko et al. *PRL* 2006

Howard: *Mechanics of Motor Proteins and the Cytoskeleton*
Sinauer Press (2001)

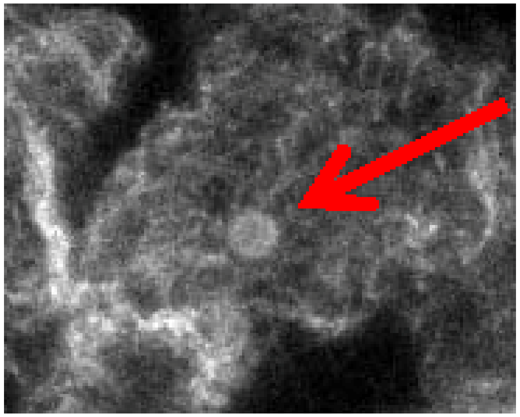
R-independence and x_β values suggest very few bonds are loaded



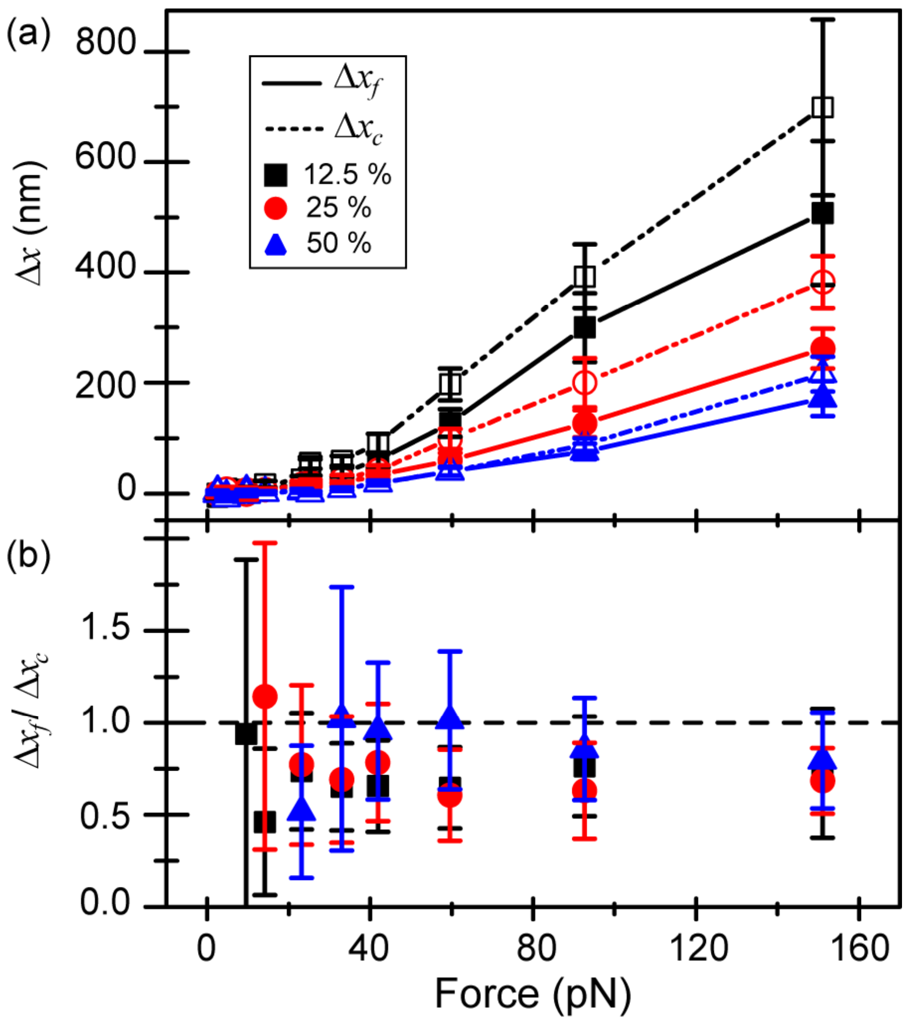
Consistent with a bond breakage mechanism

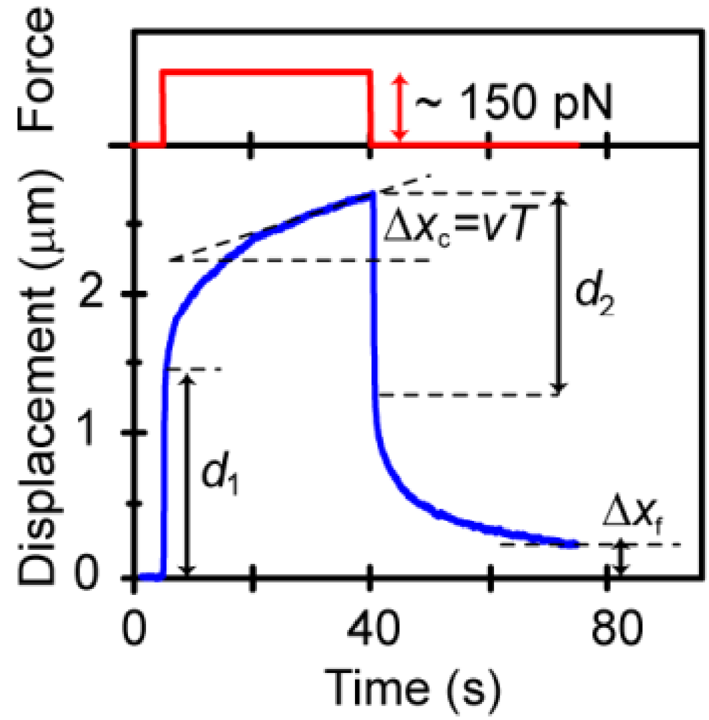
$$k_{off} = k_0 \exp(F x_\beta / k_B T)$$

F < 65 pN, x_β ~ 0.25 nm
F > 85 pN, x_β ~ 0.05 nm



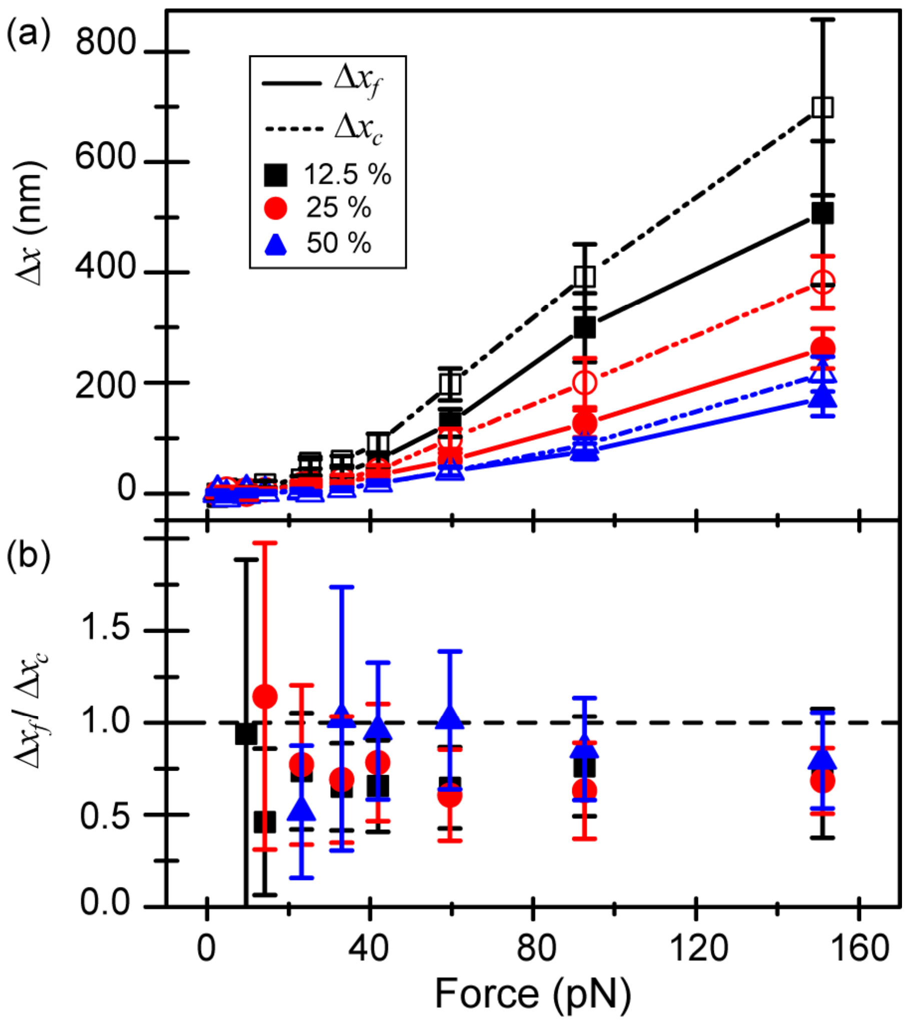
Recovery after applied stress





Bead tends to return to original position, even when plastic flow is observed due to filament rigidity.

Recovery after applied stress

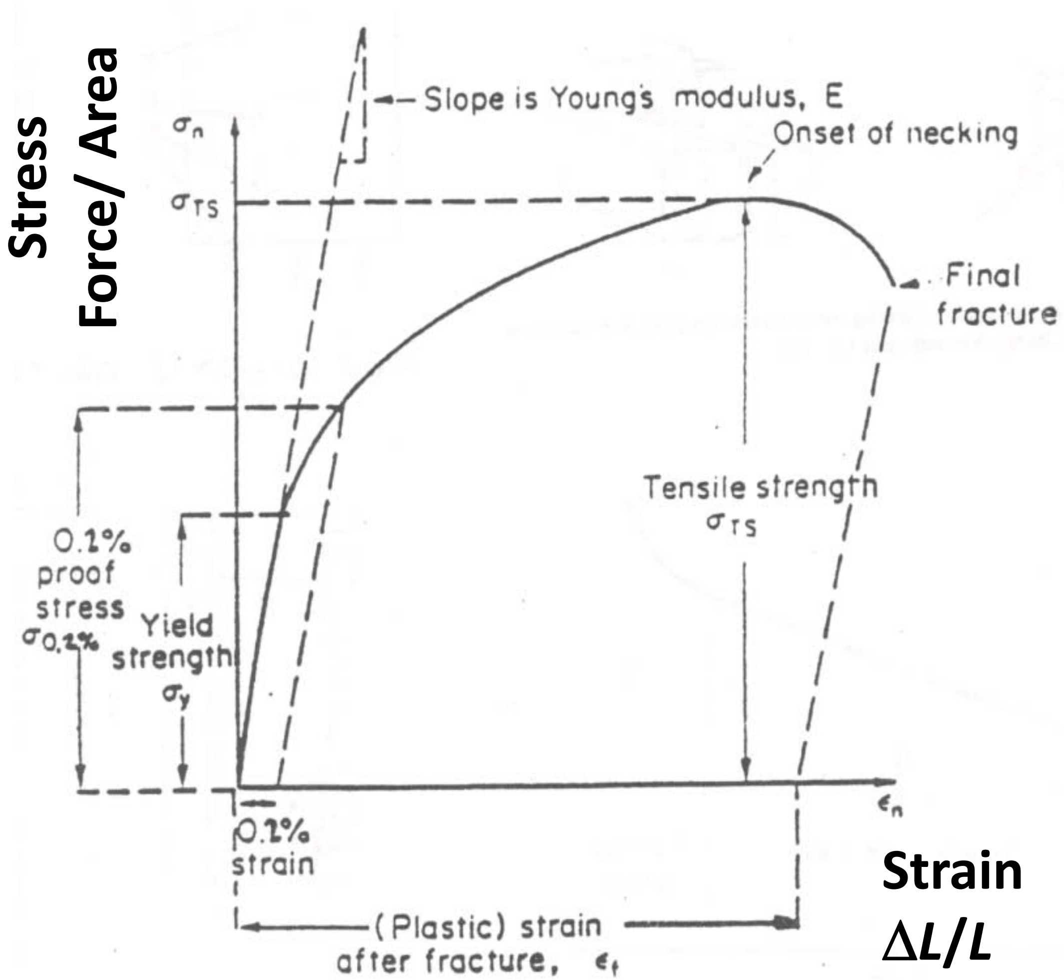


Very useful features:

- Fatigue-resistance
- Self-healing
- Toughening

Bead tends to return to original position, even when plastic flow is observed due to filament rigidity.

Toughness: dissipated energy during pull

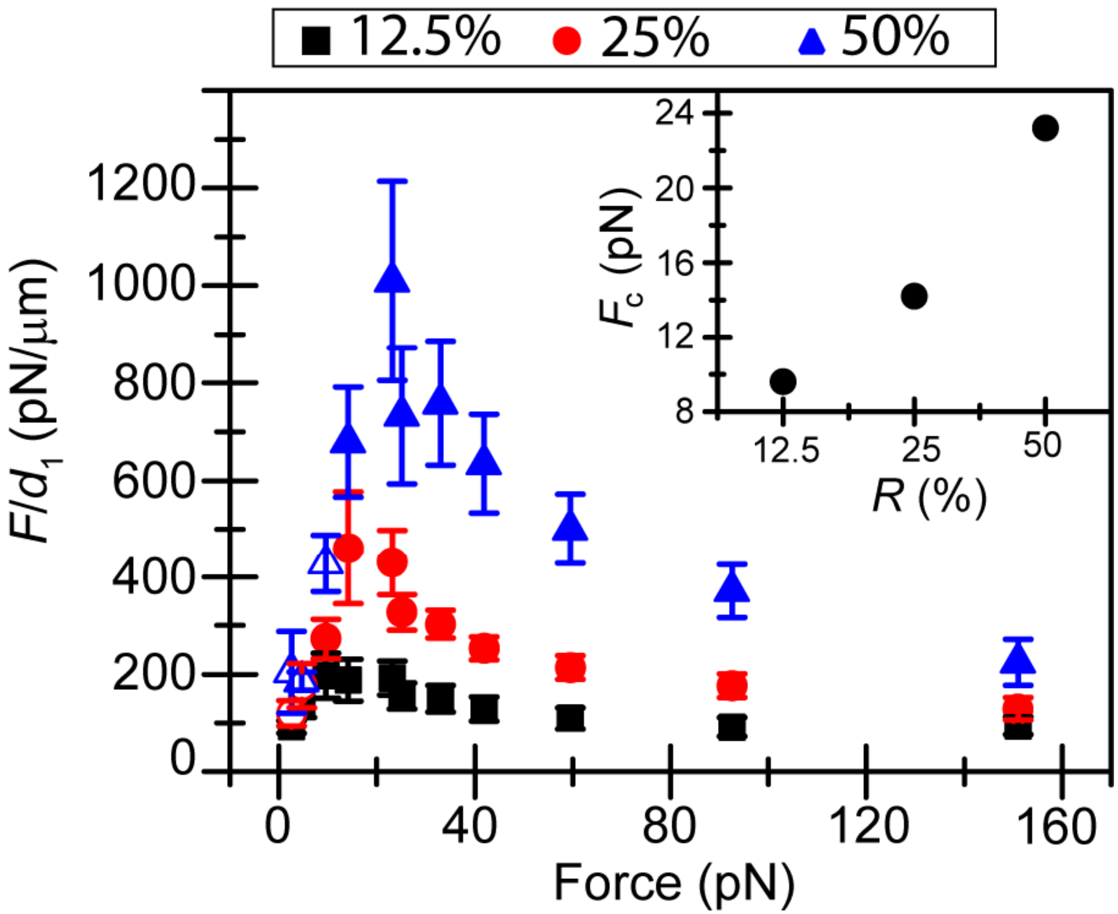


Ability of a material to ***absorb energy before fracture****.*

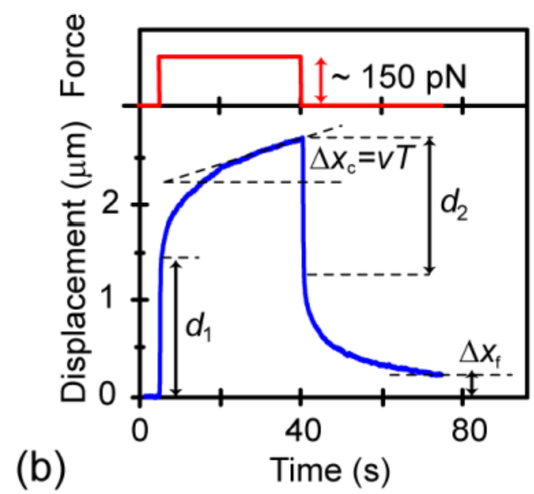
= Area under curve.

Units = Pa (Energy/Volume)

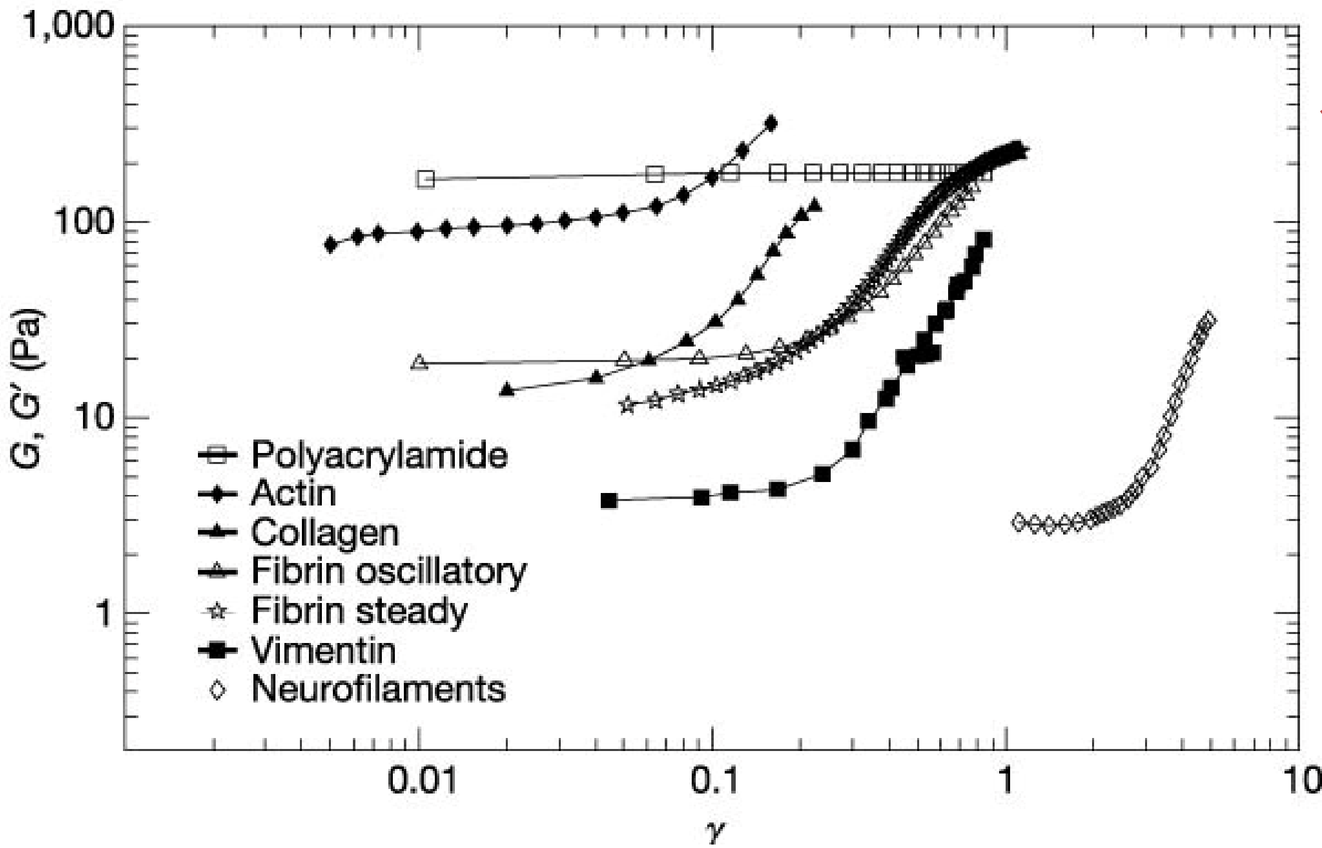
Elastic response is nonlinear with force

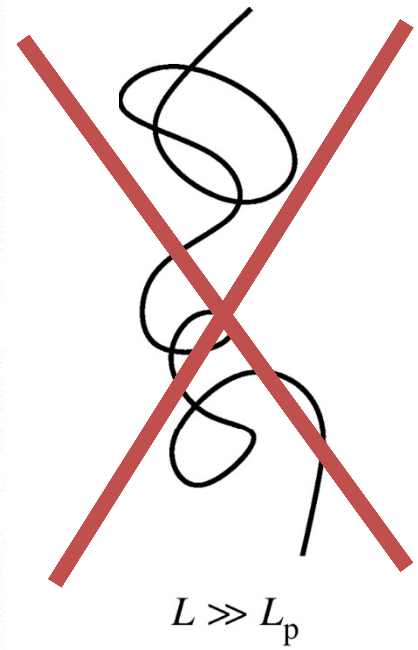


- *Stiffening at small forces*
- *Peak force and peak stiffness increase with crosslinking ratio R*
- *Softening at large forces*



Nonlinear elasticity is common in biopolymers

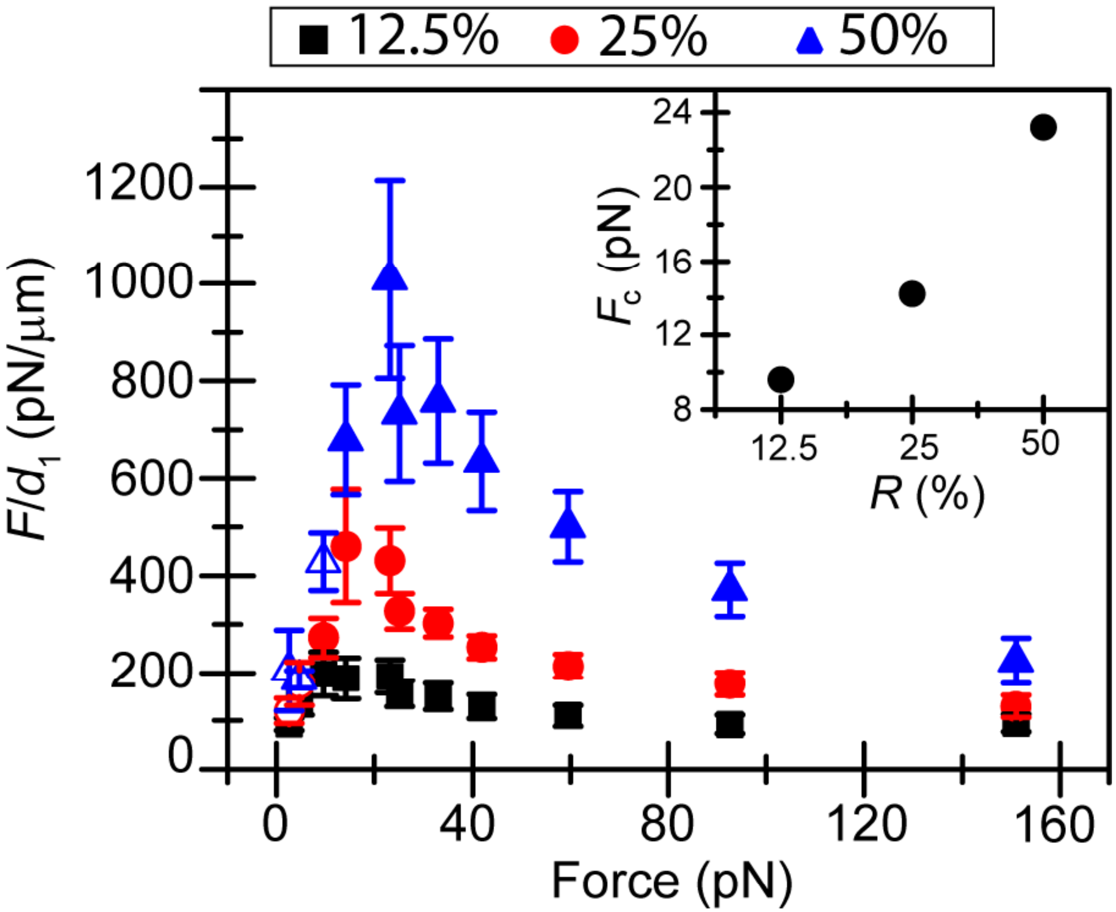




Microtubules lack excess length

Storm, et al. Nature (2005)

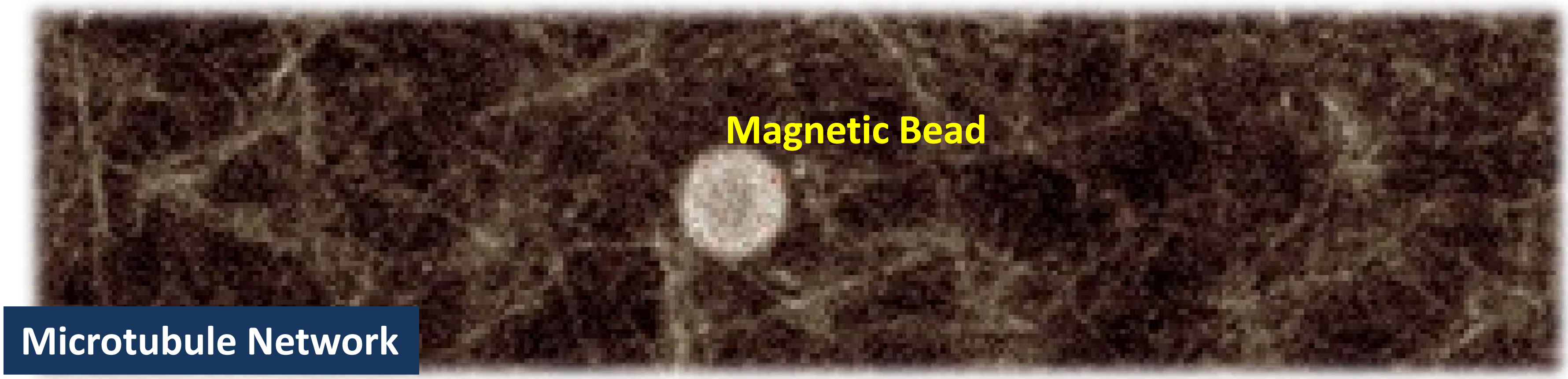
Microtubule networks are not entropic



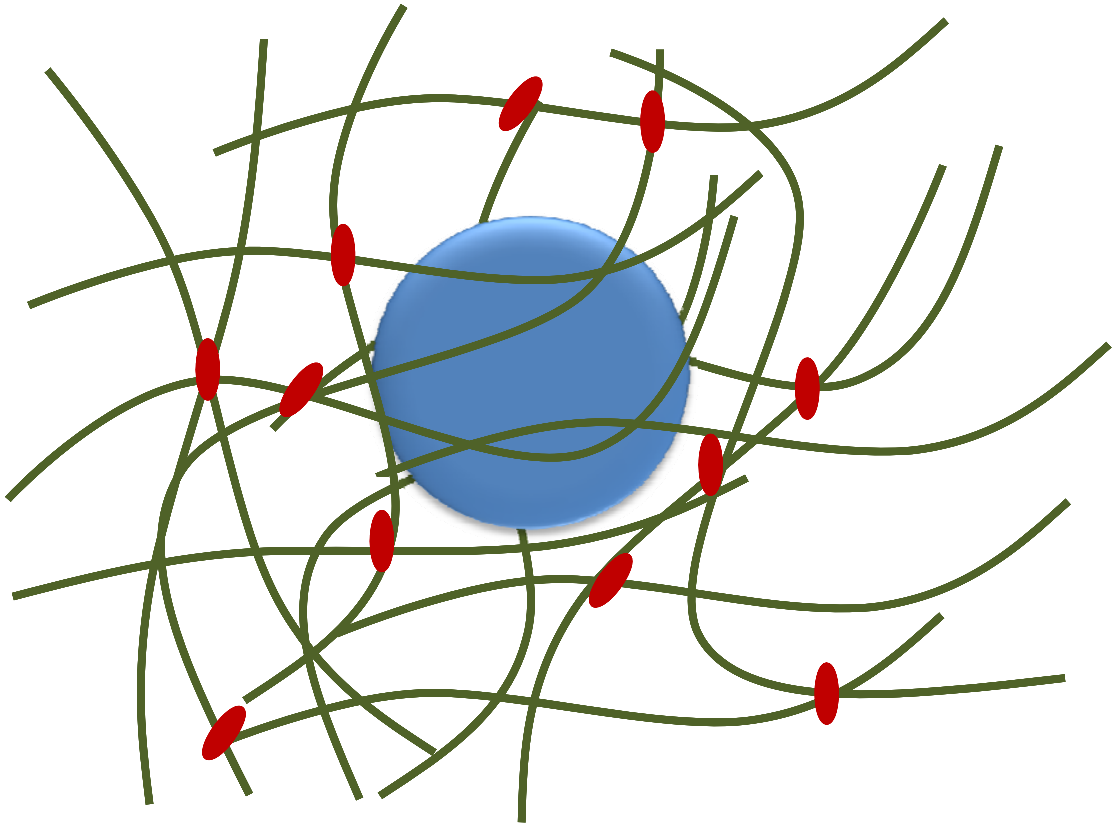
- *Stiffening due to filament alignment???*
- *Softening at large forces due to crosslinker unbinding*

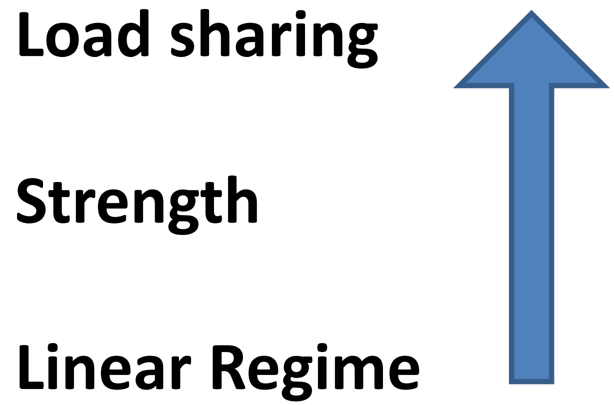
What we've learned so far….

- Microtubule networks act as **solids** subject to plastic deformation
- Bond breakage kinetics dominate time-dependent response
- Hydrodynamics and filament contour fluctuations play minor roles
- Modulation of crosslinker properties should lead to interesting new rheology, and novel materials → *also really useful for cells.*



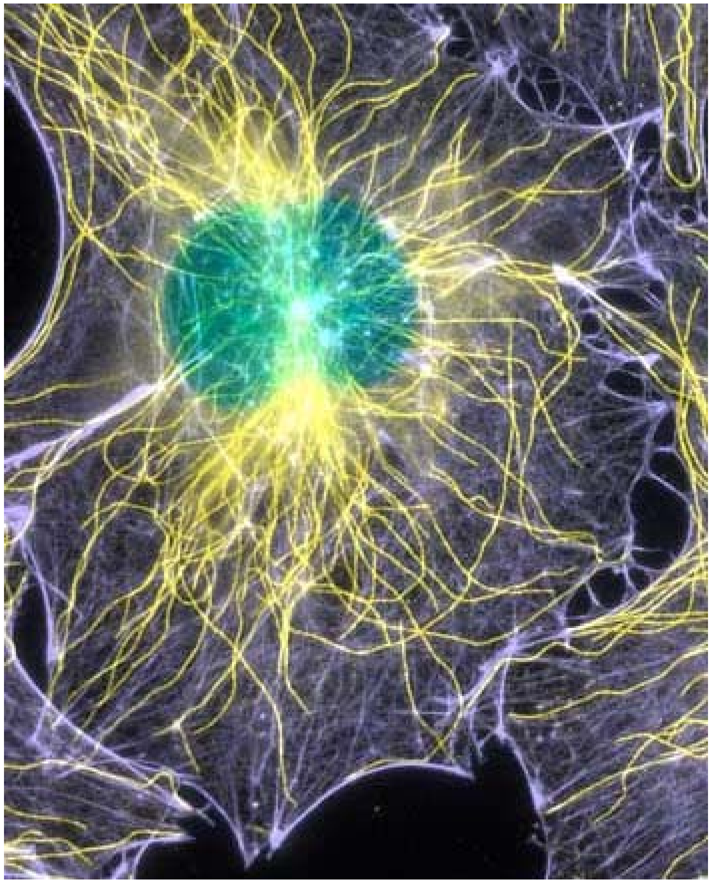
What if more compliant crosslinkers were used?





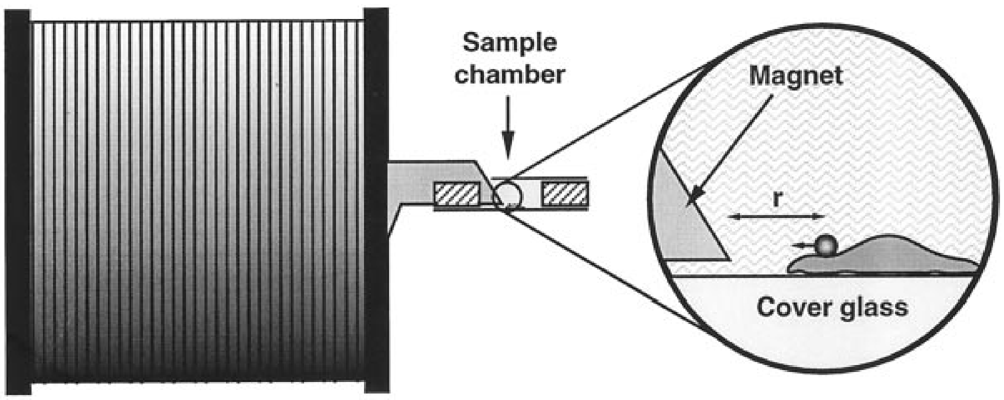
Highlights of cytoskeletal mechanics

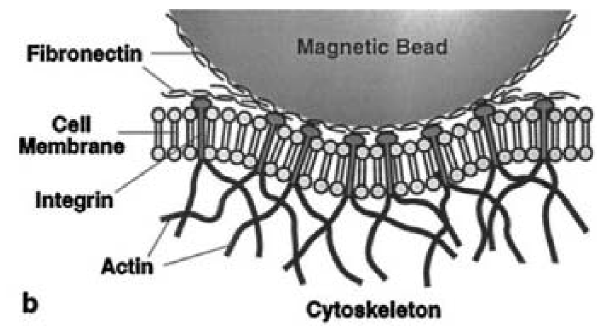
1. Cytoskeletal polymers are very stiff
2. Network mechanics is often dominated by crosslinker properties
3. **Enzymatic activity can be really important**

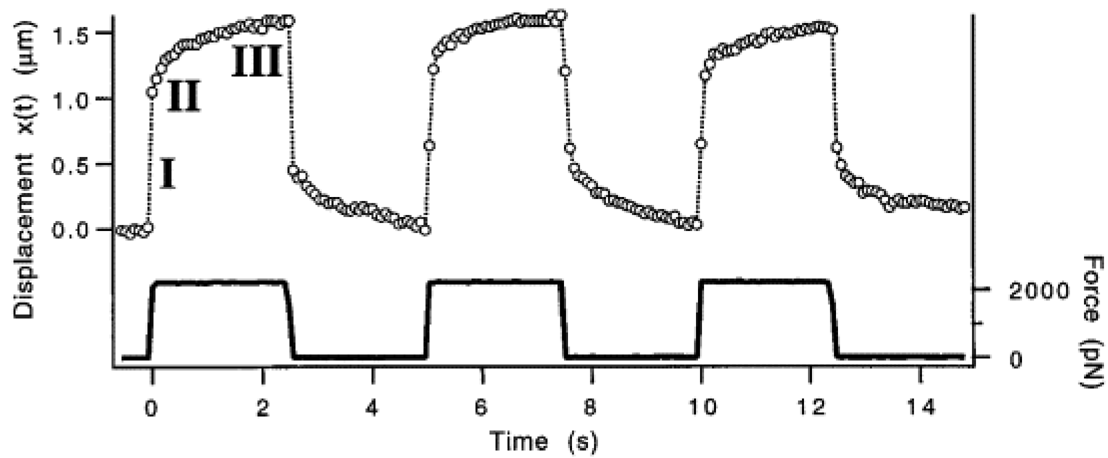


http://dir.nhlbi.nih.gov/labs/lctm/

Early studies of cell mechanics ignored this…

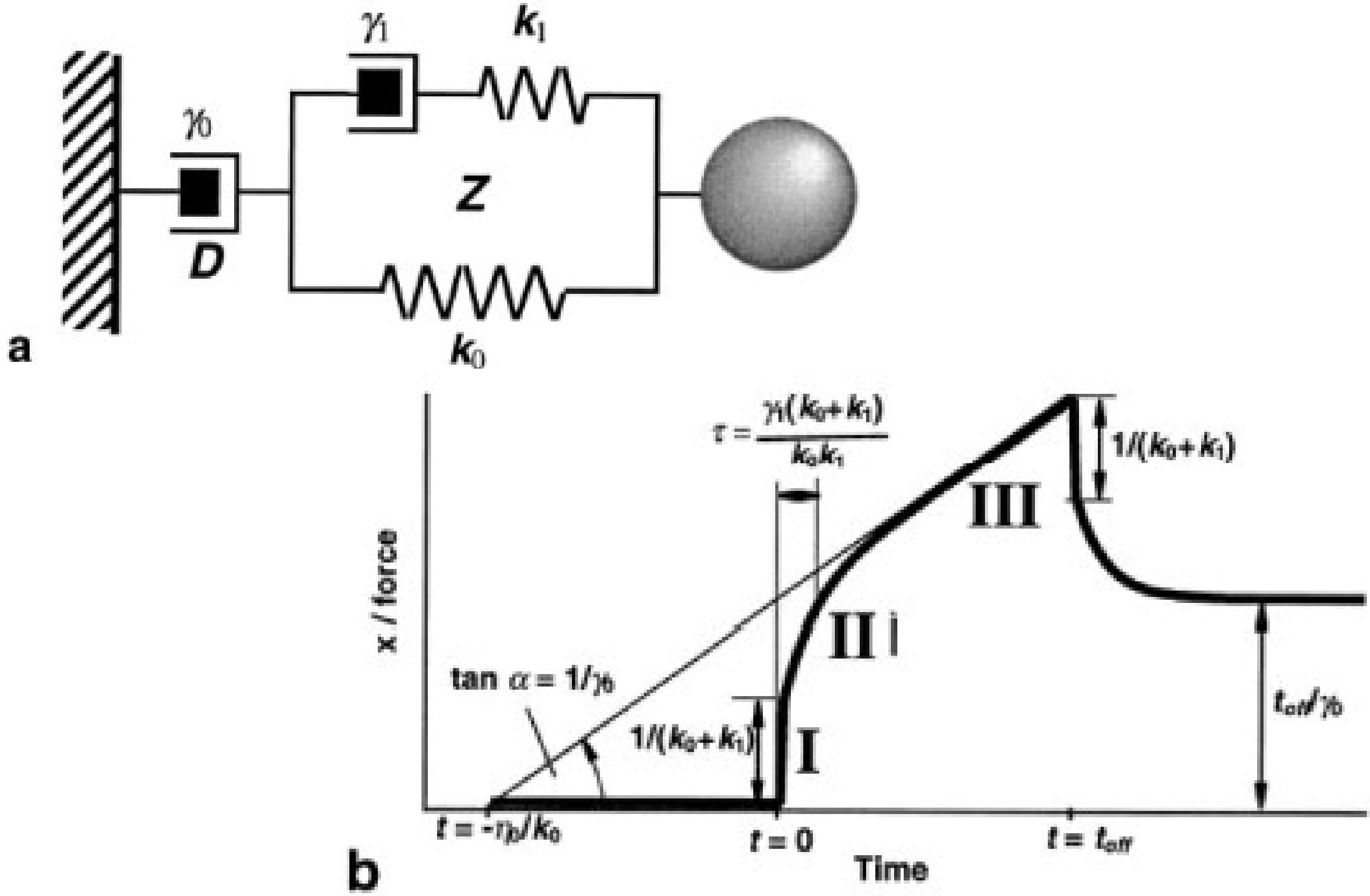




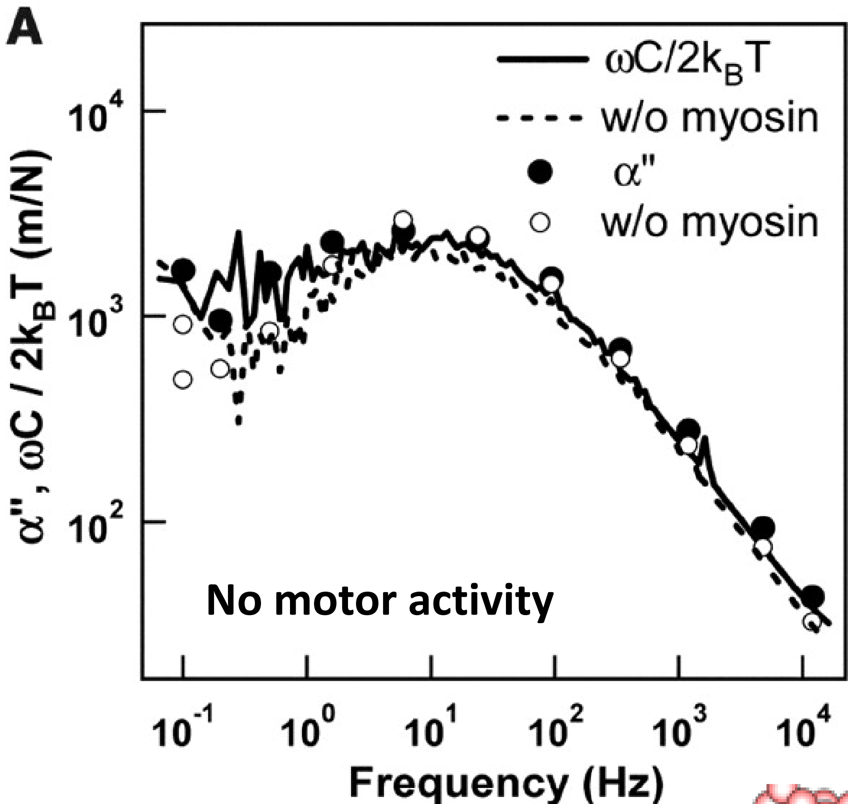


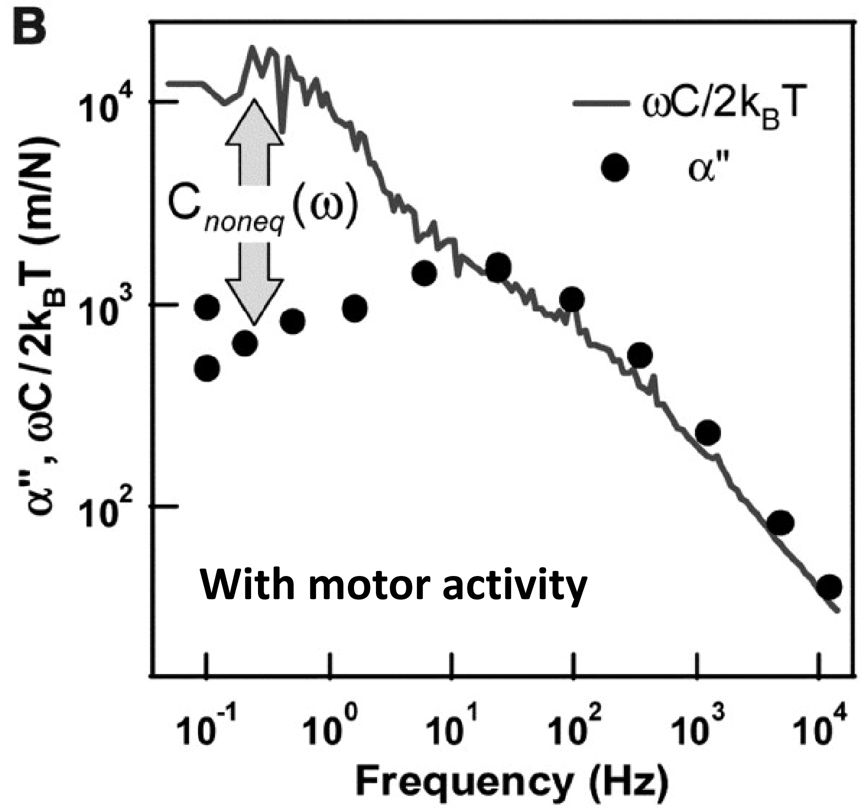
Bausch et al. Biophysical Journal (1998)

Use mechano-equivalent circuit to model

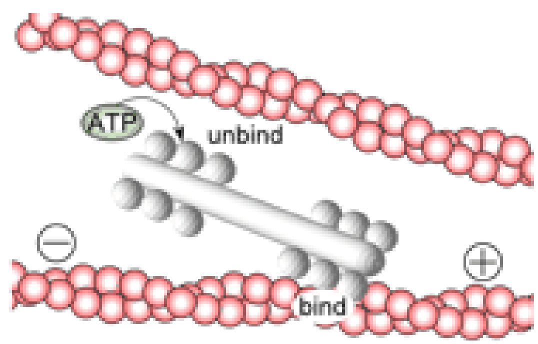


Enzymatic activity increases fluctuations





α'' → *active microrheology with oscillating optical tweezers*

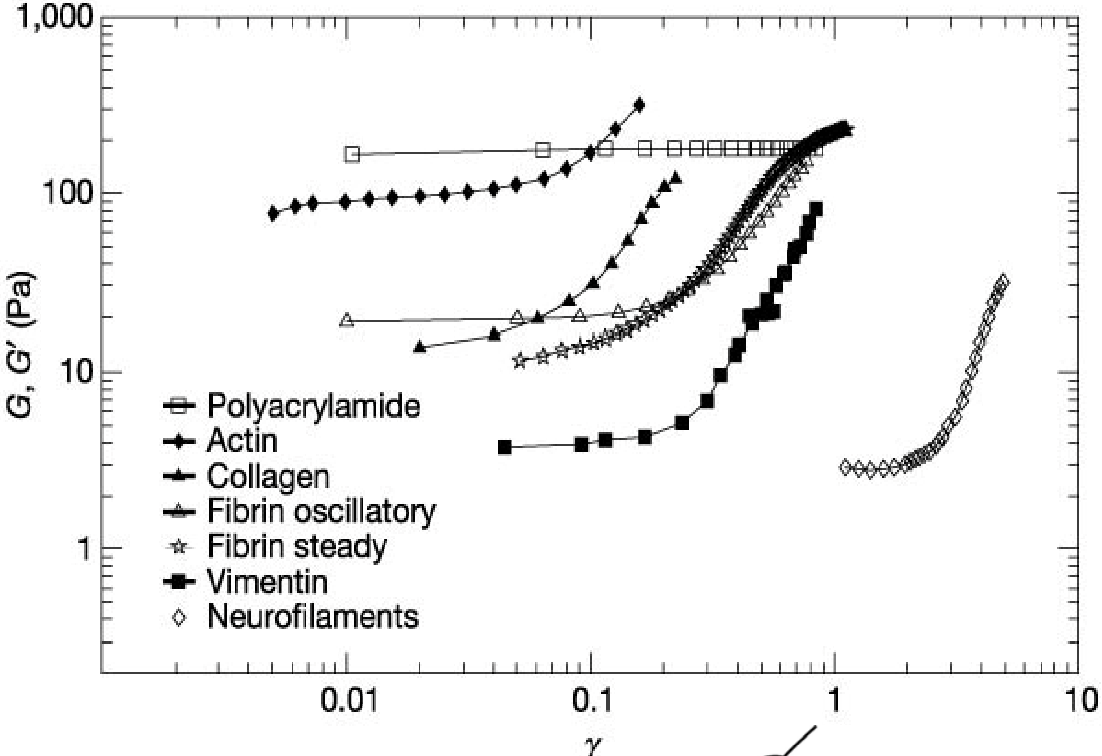


Actin

Myosin → Motors

Mizuno, et al. Science (2007)

Remember, applying tension changes stiffness…



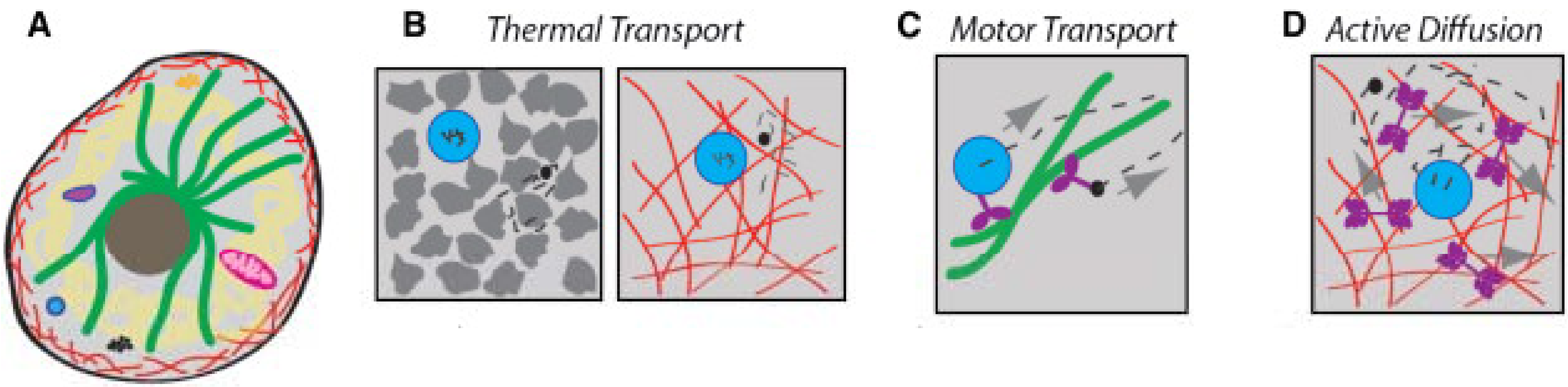
- Application of "prestress" changes network
- Motors can apply this "prestress" to tune network properties

Gardel, et al. PNAS (2006)
Koenderink, et al. PNAS (2009)

"Excess Length"

Storm, et al. Nature (2005)

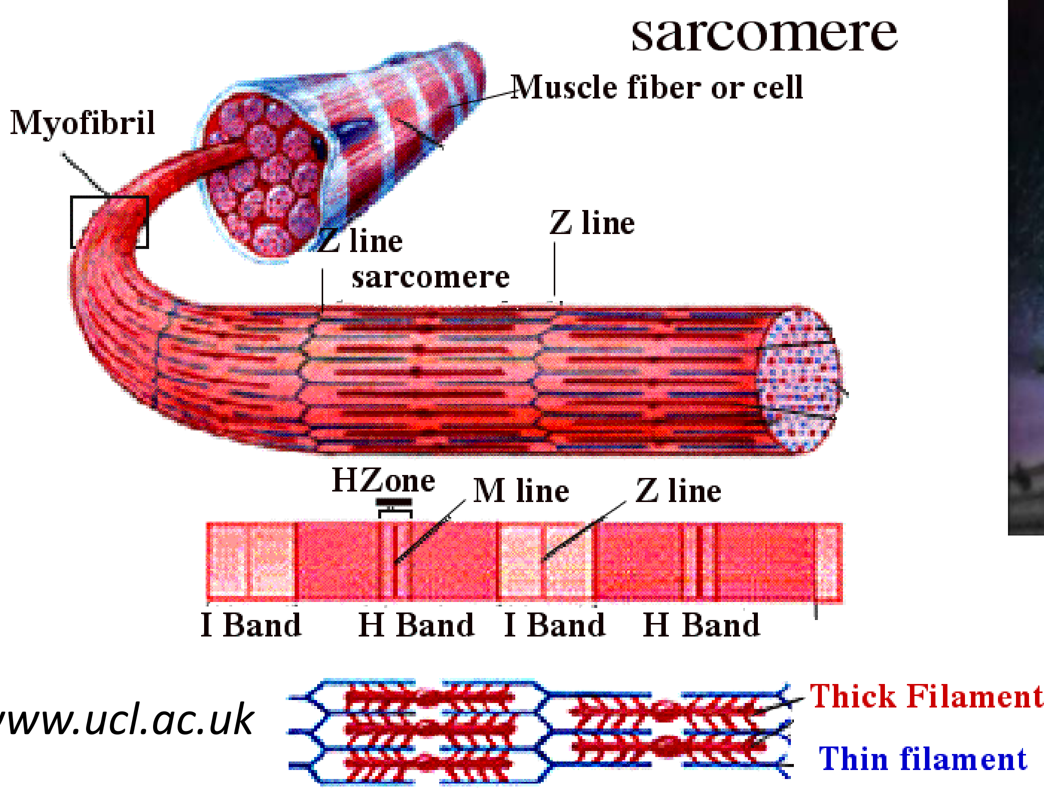
Effects of Cell Activity on Rheology



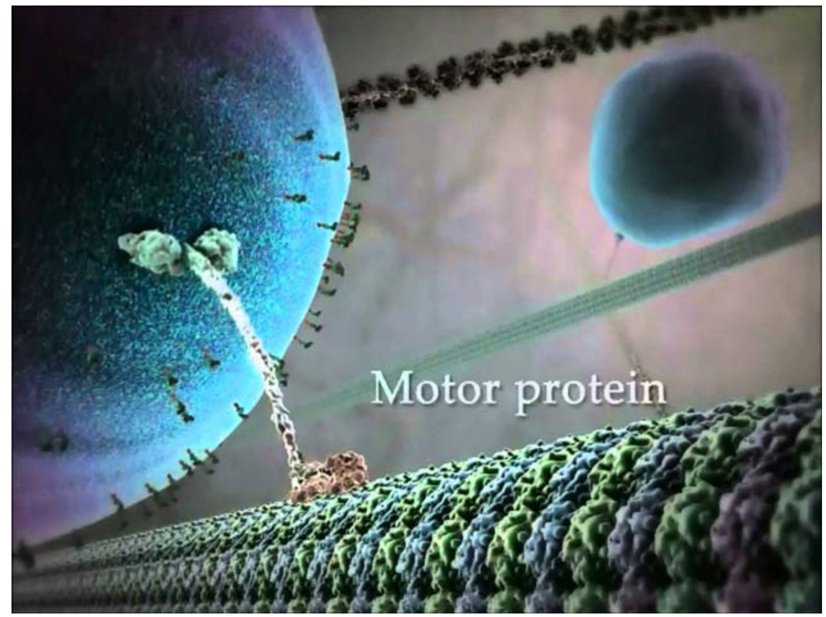
Guo et al., Cell (2014)

Stam and Gardel, Developmental Cell **30**(4) p. 365-366 (2014)

Motor Proteins

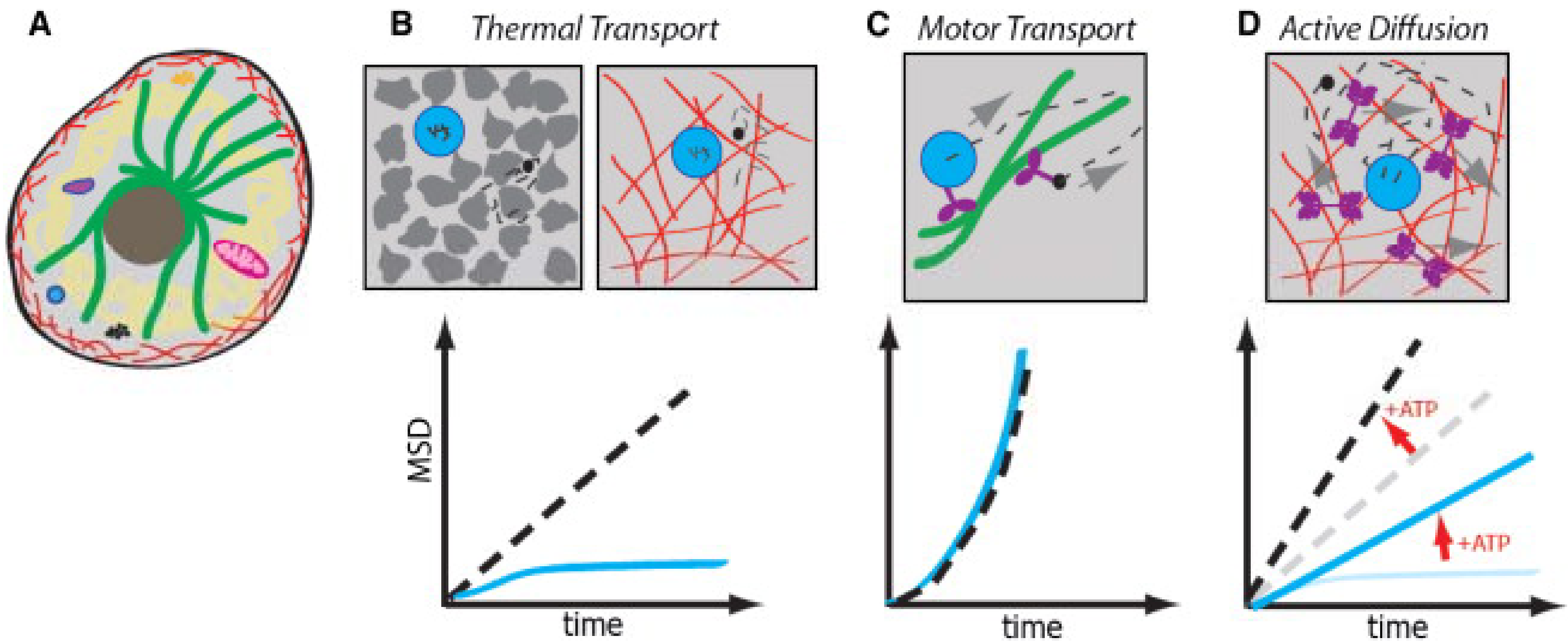


www.ucl.ac.uk



Convert chemical energy into mechanical work

Effects of Cell Activity on Rheology



Guo et al., Cell (2014)

Stam and Gardel, Developmental Cell **30**(4) p. 365-366 (2014)

Bottom Line…

- Microrheology methods are excellent means of characterizing soft biomaterials

- Entangled polymer theories have limited usefulness in understanding cell mechanics, but concepts from polymer physics and statistical mechanics are essential

- We still understand very little about cell mechanics, but now have a decent set of theoretical and experimental tools

- Connecting physical models (often phenomenological) to detailed molecular mechanisms remains a challenge.