Microrheology, motors, fluctuations

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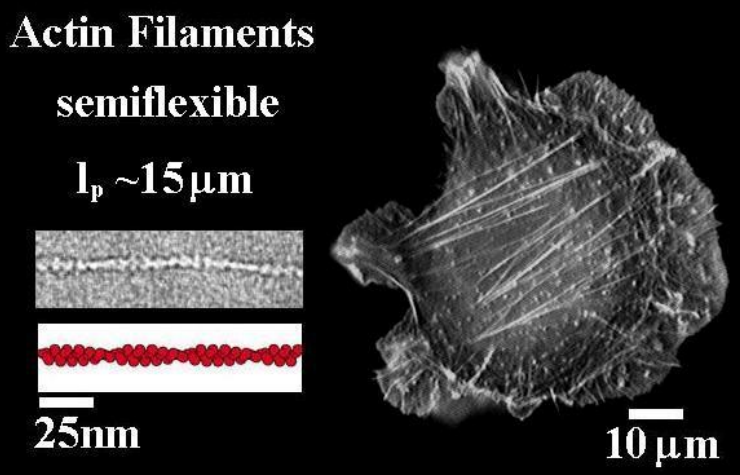
Angelo Mao	Harvard	Gijsje Koenderink	FOM

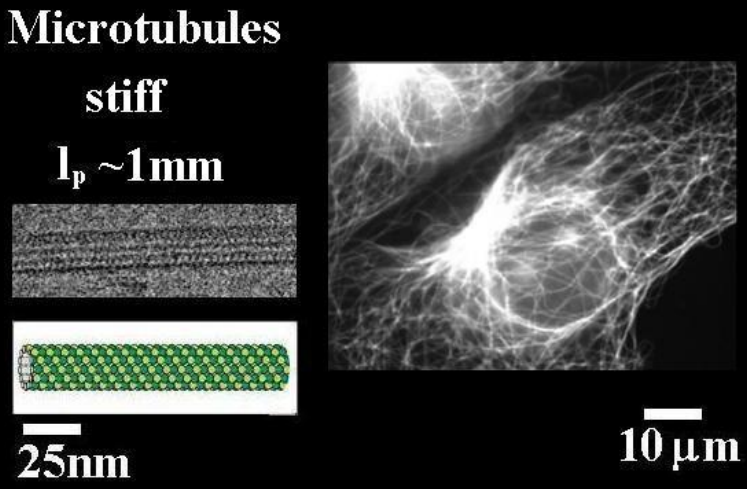
1. Non-linear elasticity of actin networks
2. Effect of molecular motors
3. Fluctuations of microtubules
4. Fluctuations within cells

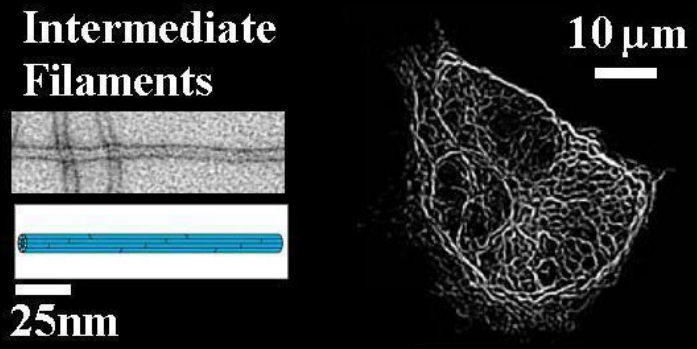
http://weitzlab.seas.harvard.edu/

Active Systems
GIST, 6/26/14

Biopolymer networks provide mechanical rigidity



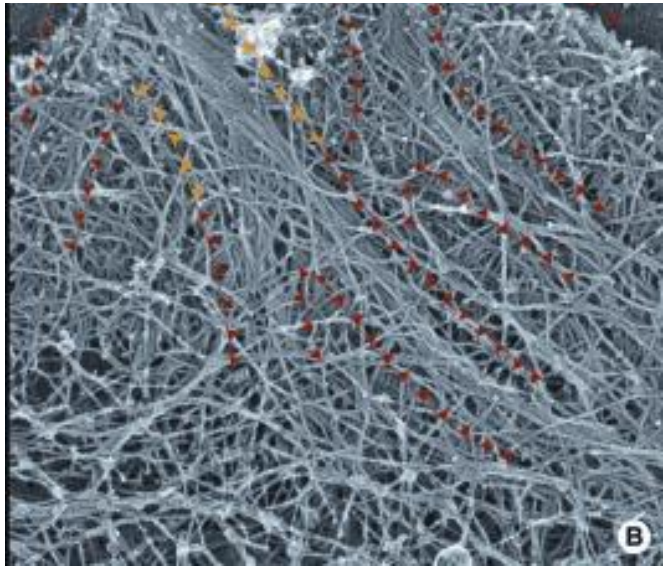


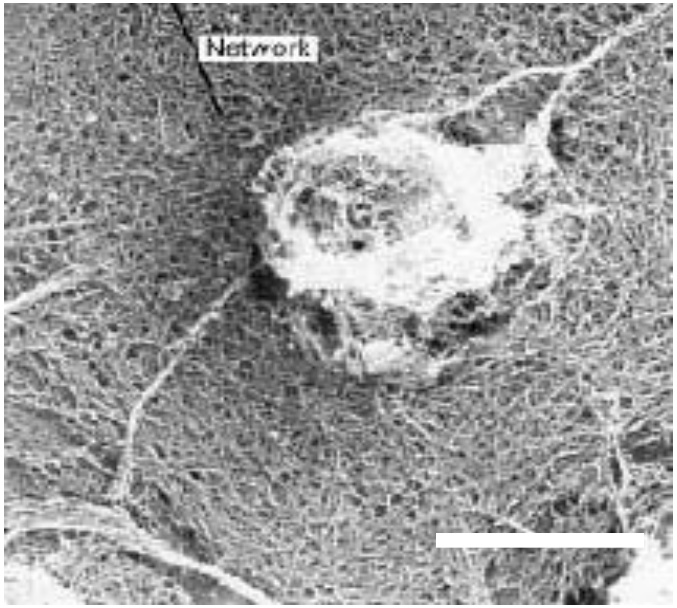


- Cytoskeleton crowds the cell
  - network meshsize << 1μm
- Total protein volume fraction in
  - cells is typically 20-30%

Actin Networks

Study reconstituted networks

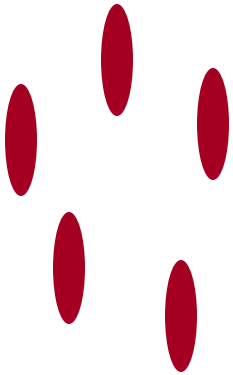




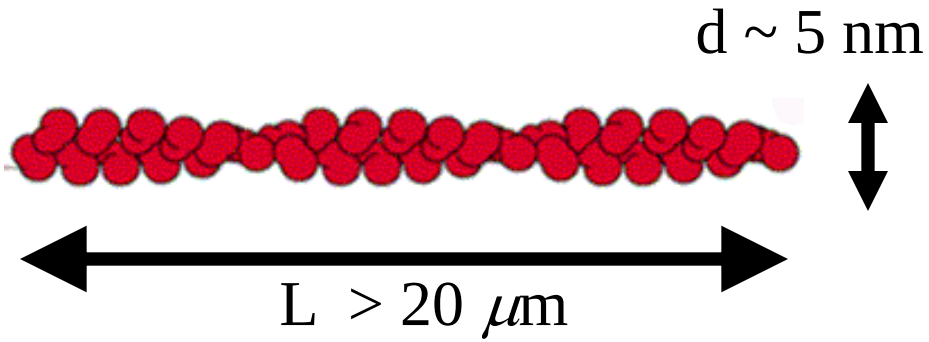
5 μm

Actin filaments

G-actin



Polymerize in presence of ATP, divalent salt
Mass ~ 42k Da
a ~ 7 nm

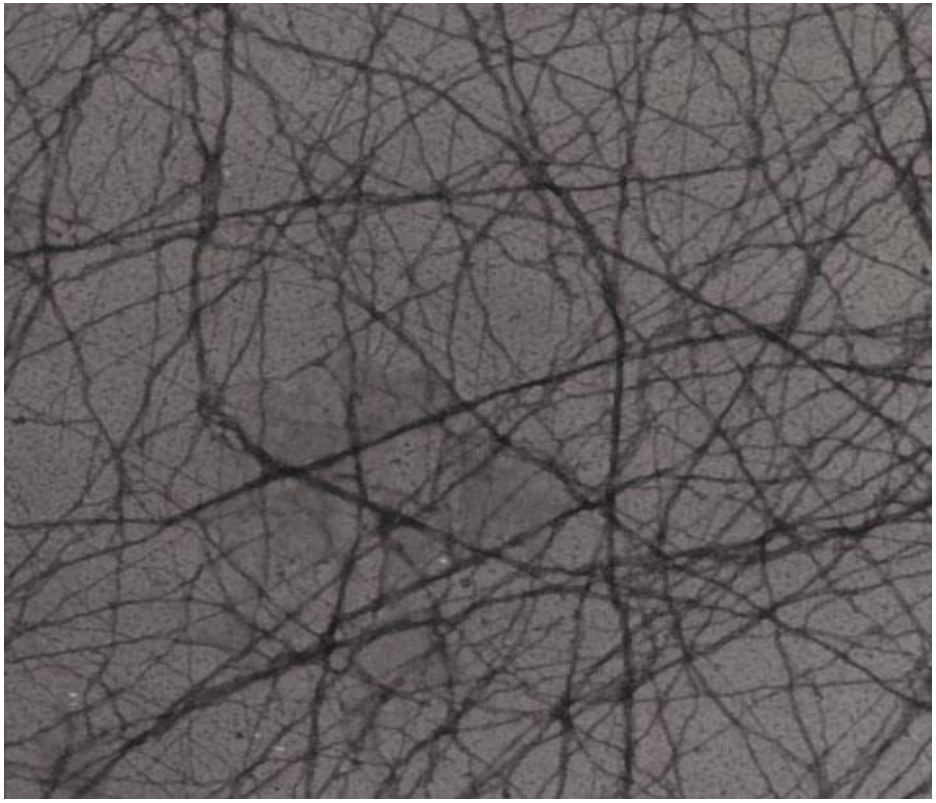


- Young's Modulus, $E \sim 10^9$ Pa
  → Hard Plastic
- Large Aspect Ratio $d << L$
  → Soft Bending Modulus

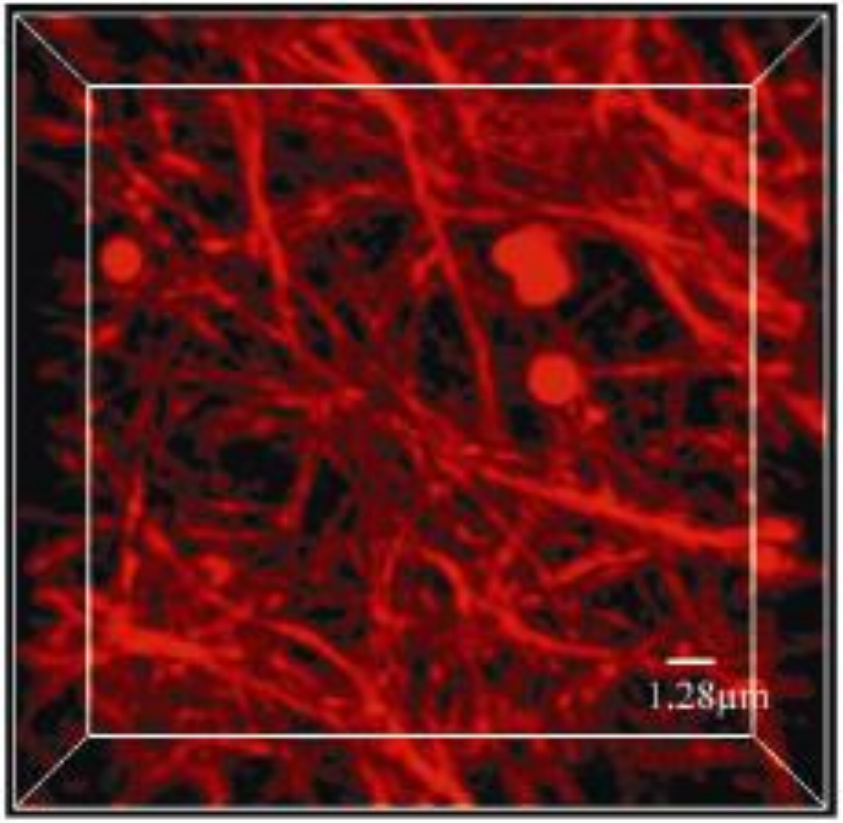
$$\kappa \sim Ed^4$$

- 1-5% of proteins in nonmuscle cells

Reconstituted Actin Networks

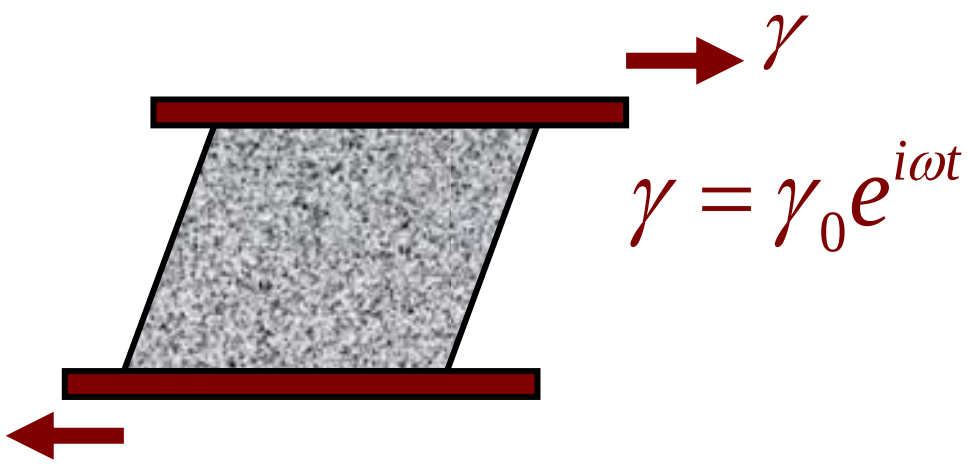


Actin network
Electron Microscopy



Actin with bundling protein
Confocal Microscopy

Viscoelasticity of Soft Materials



Solid: $\tau = G\gamma$

Fluid: $\tau = \eta\dot{\gamma}$

$$\tau = \left[G'(\omega) + iG''(\omega)\right]\gamma$$

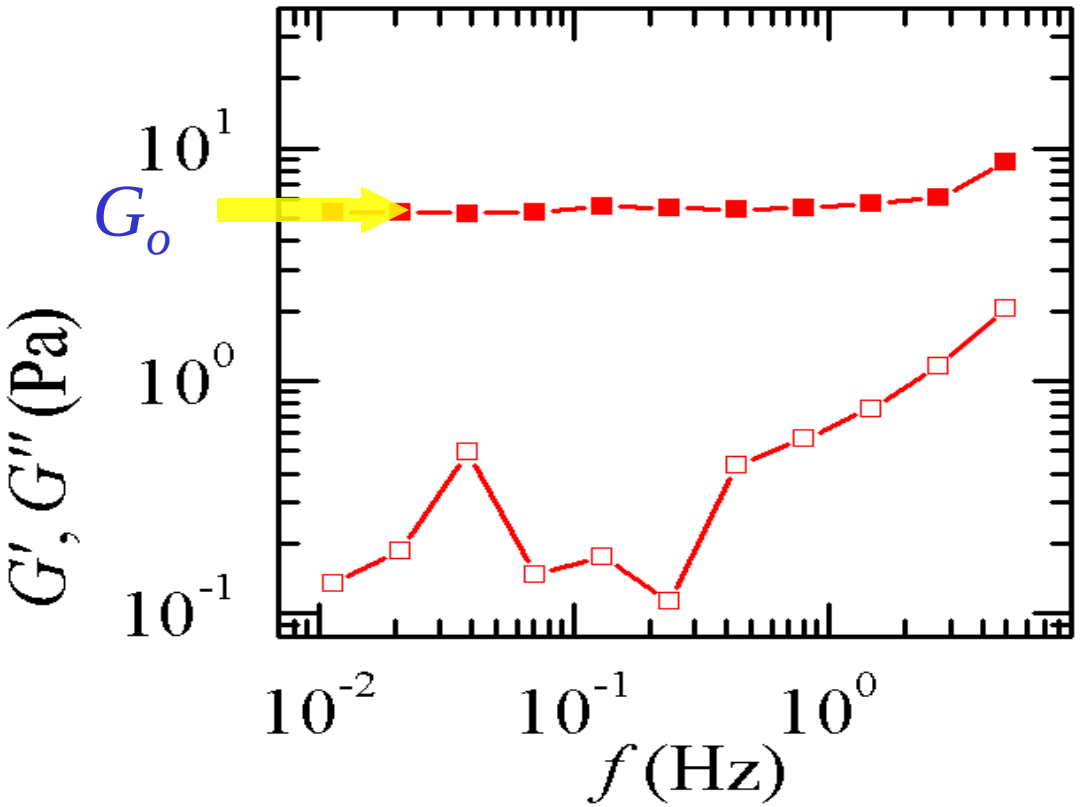
Elastic

Viscous

Assumes intrinsically equilibrium system → causality (Kramers-Kronig)

Linear Elastic Response

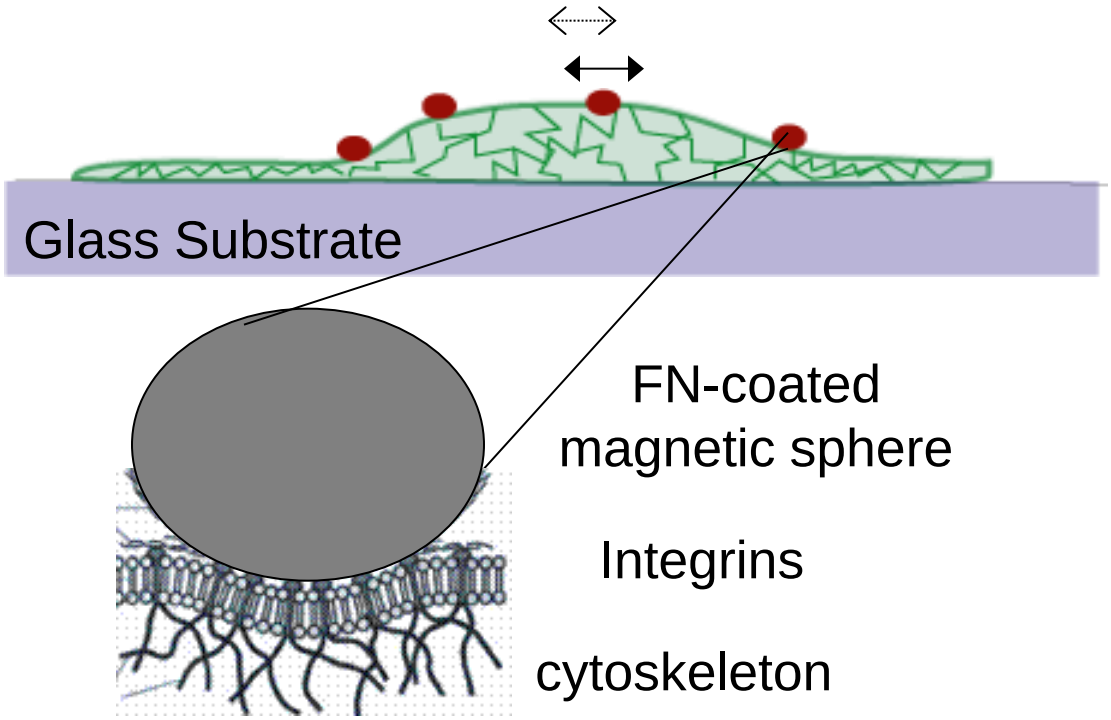
■ Elastic, G' □ Viscous, G''



c_A=12 μM R=0.03

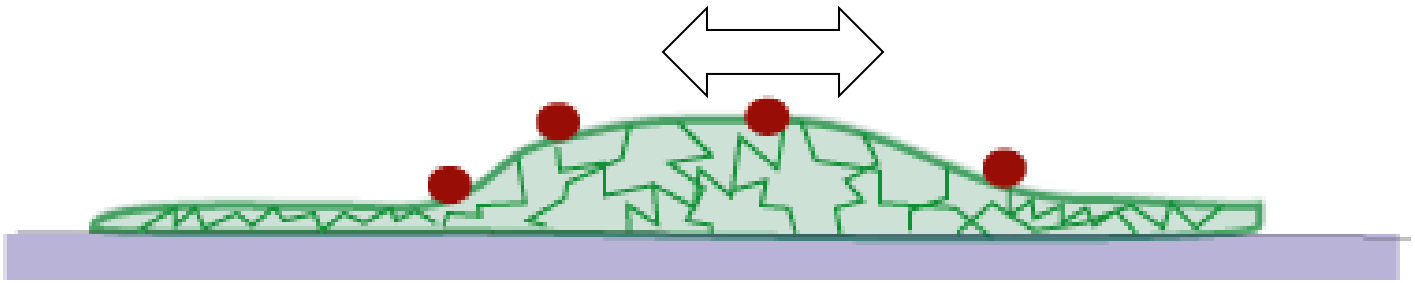
weak, elastic gel

Cytoskeletal Mechanics

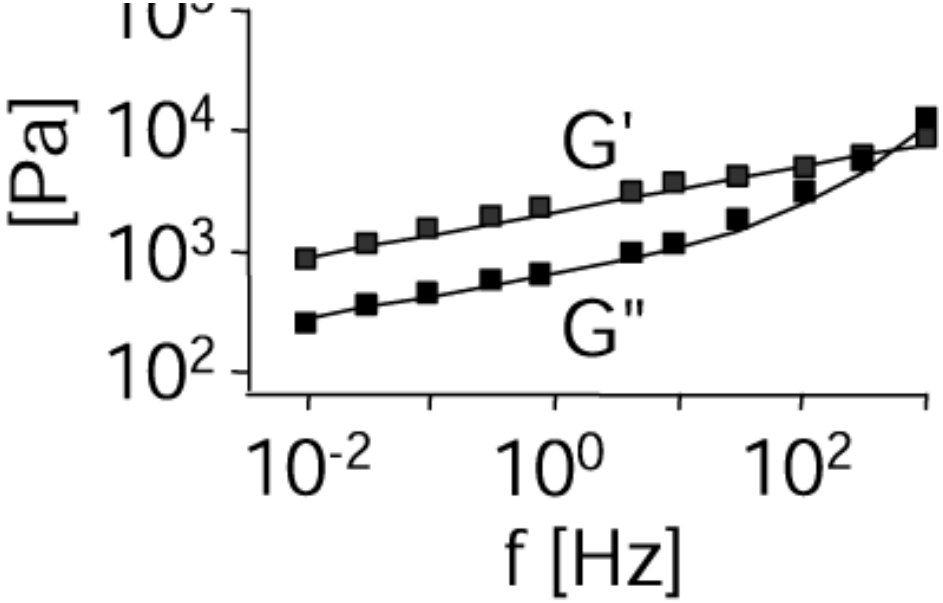


Bausch et. al., Fabry et. al.

Rheology of cells: twisting-beads

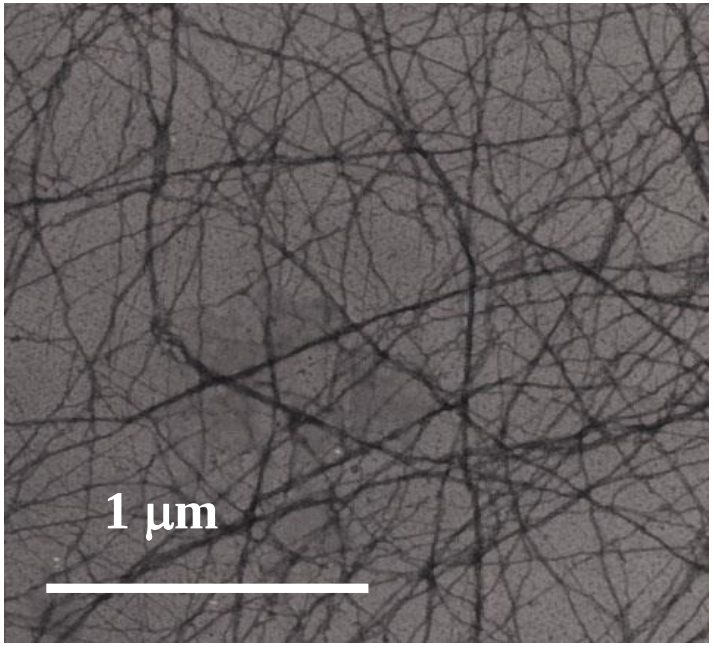


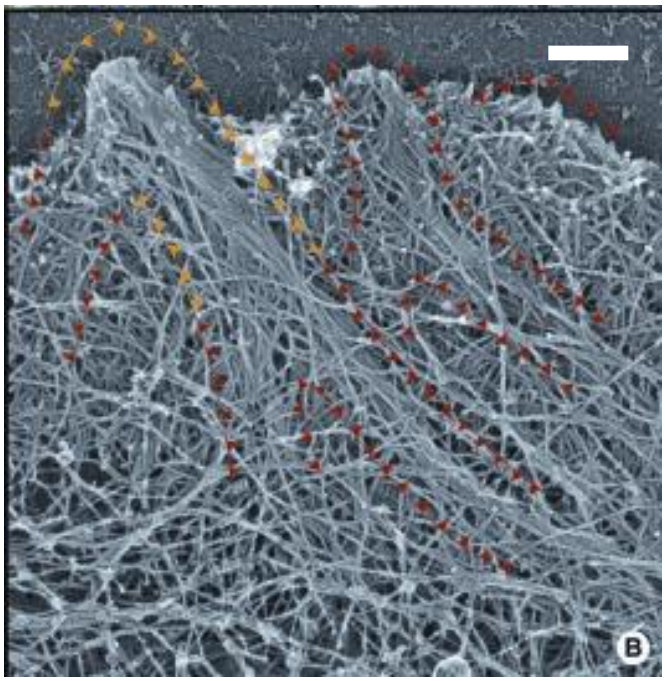
Cells are much stiffer than actin networks



(Fabry, Fredberg, 2003)

Actin Networks





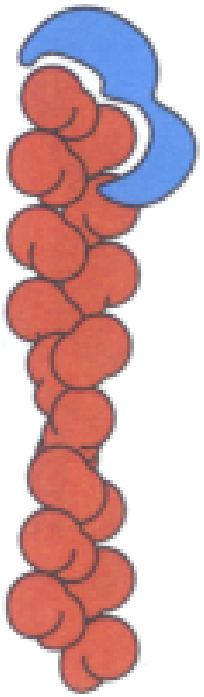
in vitro

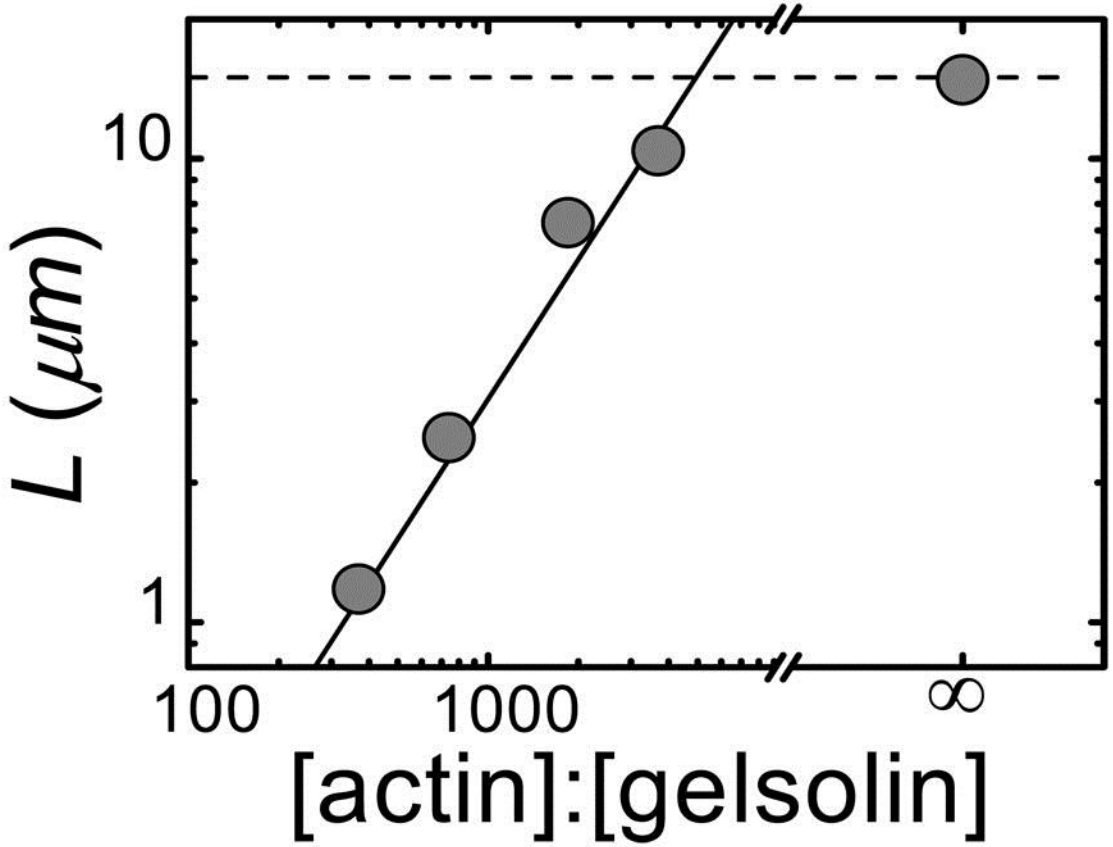
in vivo

Filaments are shorter

Filaments are cross-linked

Gelsolin → Capping

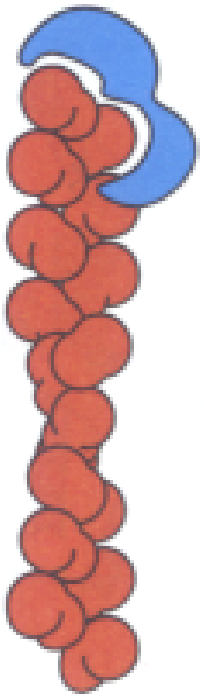


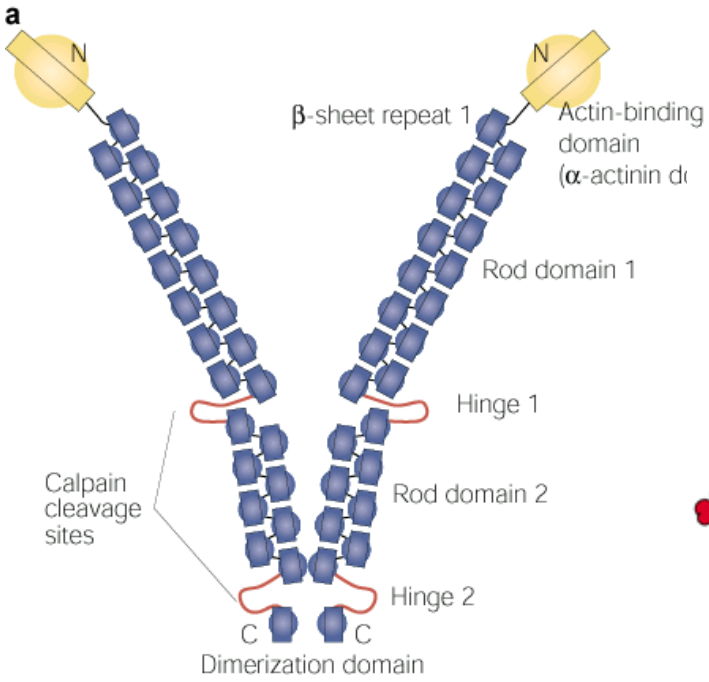


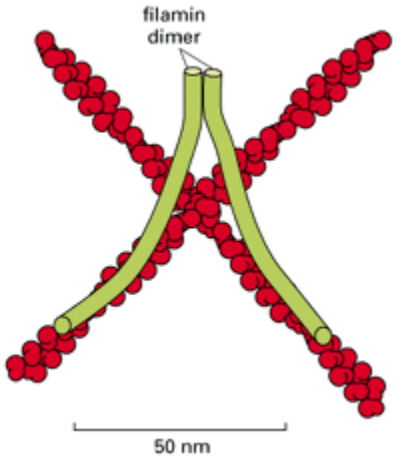
Limits filament length

Gelsolin → Capping

Filamin → Cross-linking





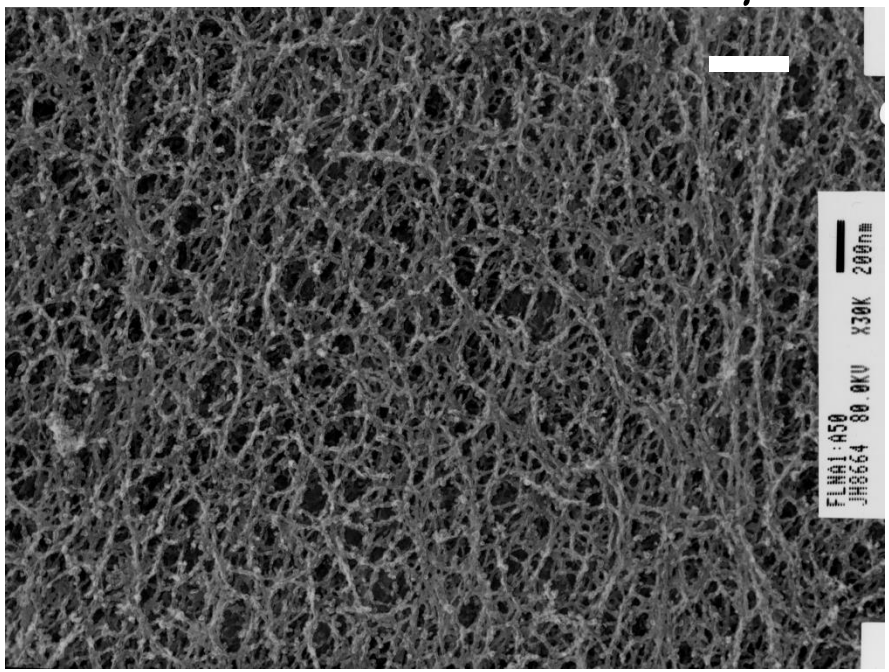


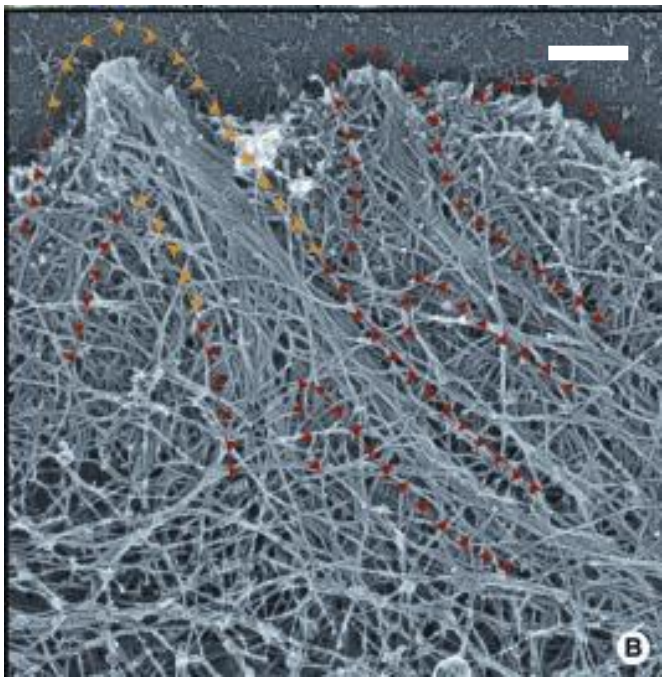
Limits filament length

Cross-links filaments
Cell motility, mechanoprotection

Actin Networks

Filamin



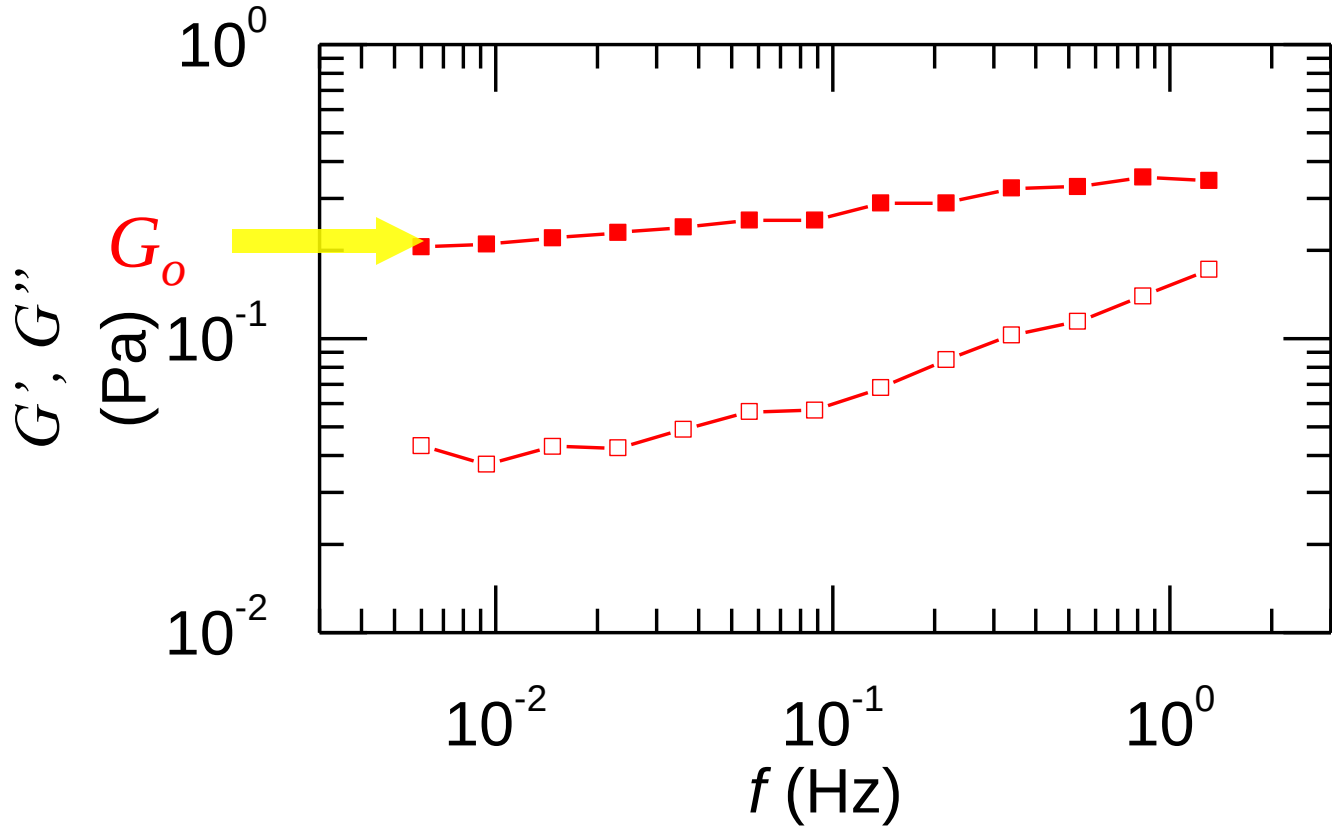


in vitro

in vivo

Actin (24 μM), 1/50 FlnAwt, 1/555 gelsolin,
MgATP (5 mM), KCl (50 mM)

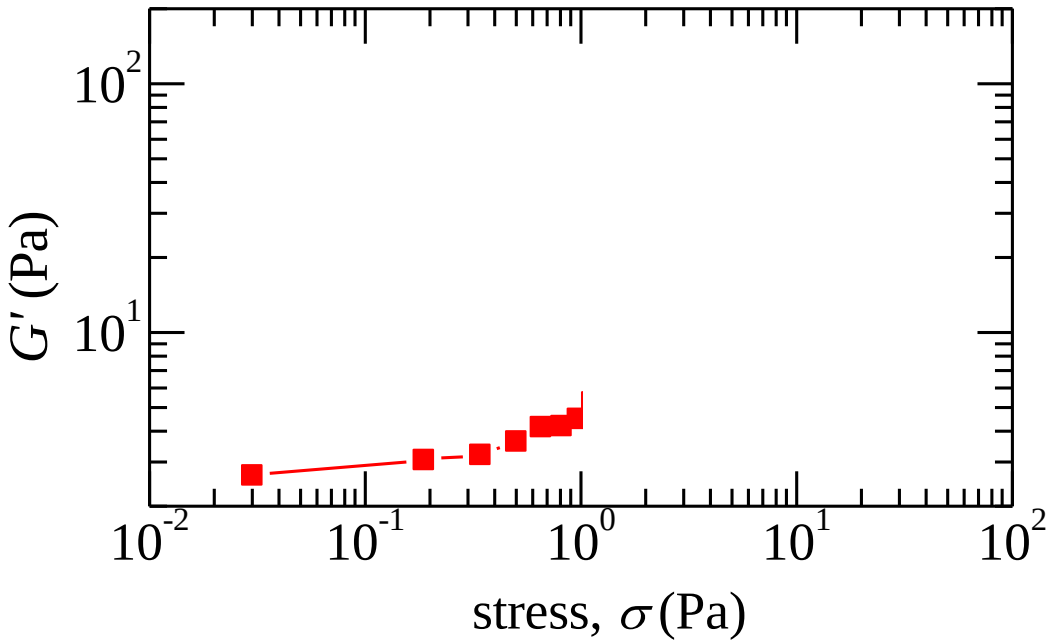
Actin-filamin networks are gels



2.2 mg/mL 100:1 1 micron

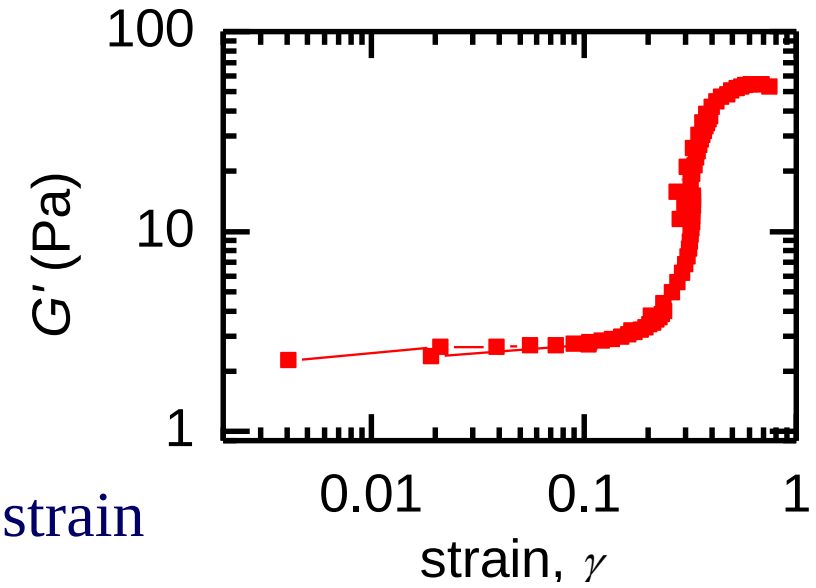
Weak elastic gel → G_0

G' extremely sensitive to stress



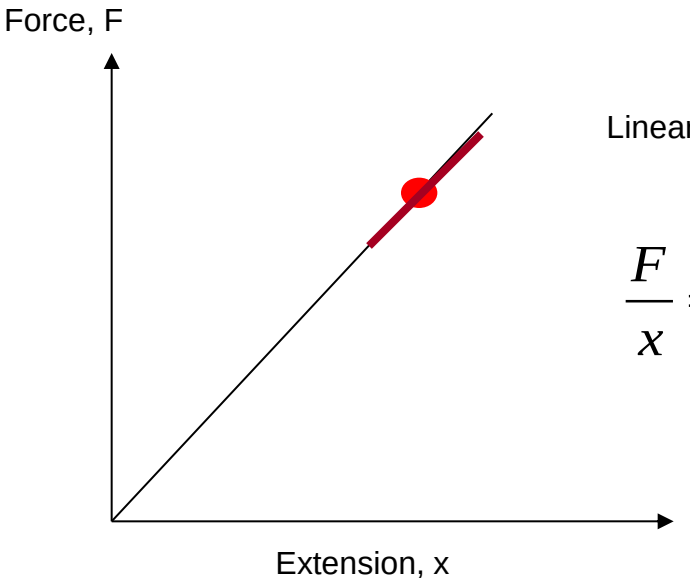
$$G'\gamma \sim \sigma$$

Breaking Stress > 80 * *G'*

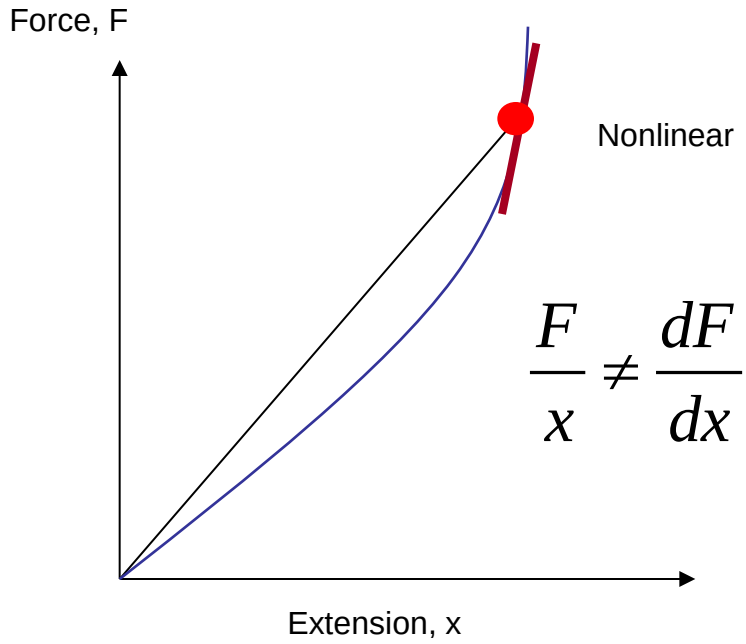


Stiffens over 40-fold with no change in strain

Non-linear spring constant



$$\frac{F}{x} = \frac{dF}{dx}$$

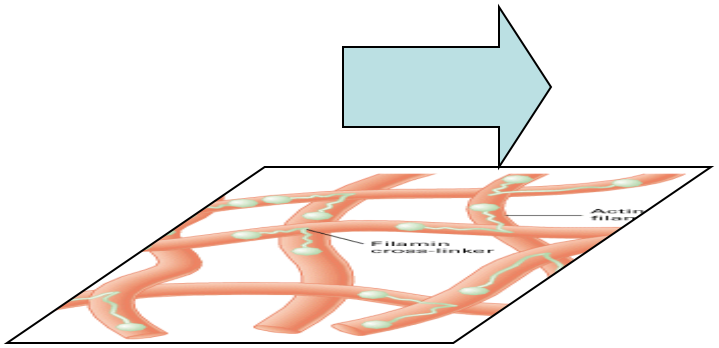


$$\frac{F}{x} \neq \frac{dF}{dx}$$

Need new ways to measure elasticity

Measure differential spring constant

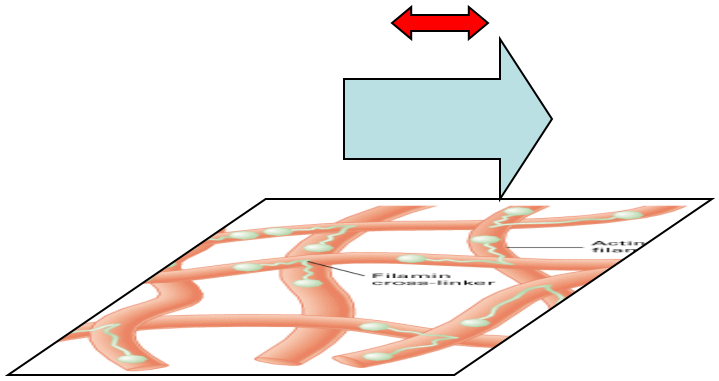
Linear Measurements in Nonlinear Regime



Creep Test:

Apply Steady Stress: σ_s

Measure γ

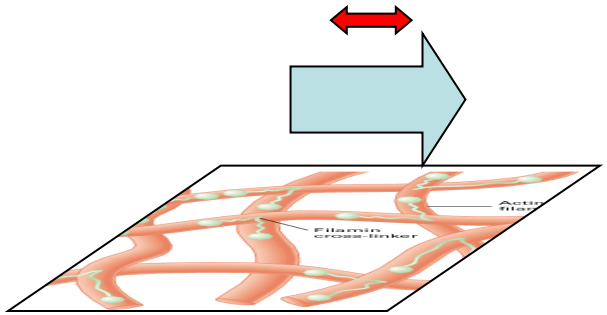


Differential Modulus:

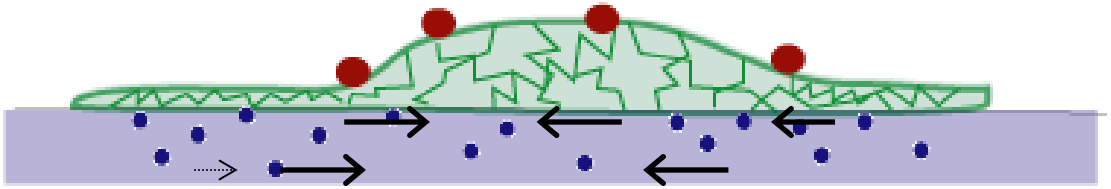
$$\sigma(t)=\sigma_s+\sigma_o \sin(\omega t)$$

Measure $\delta\sigma(\omega)/\delta\gamma(\omega)=K'(\omega)$

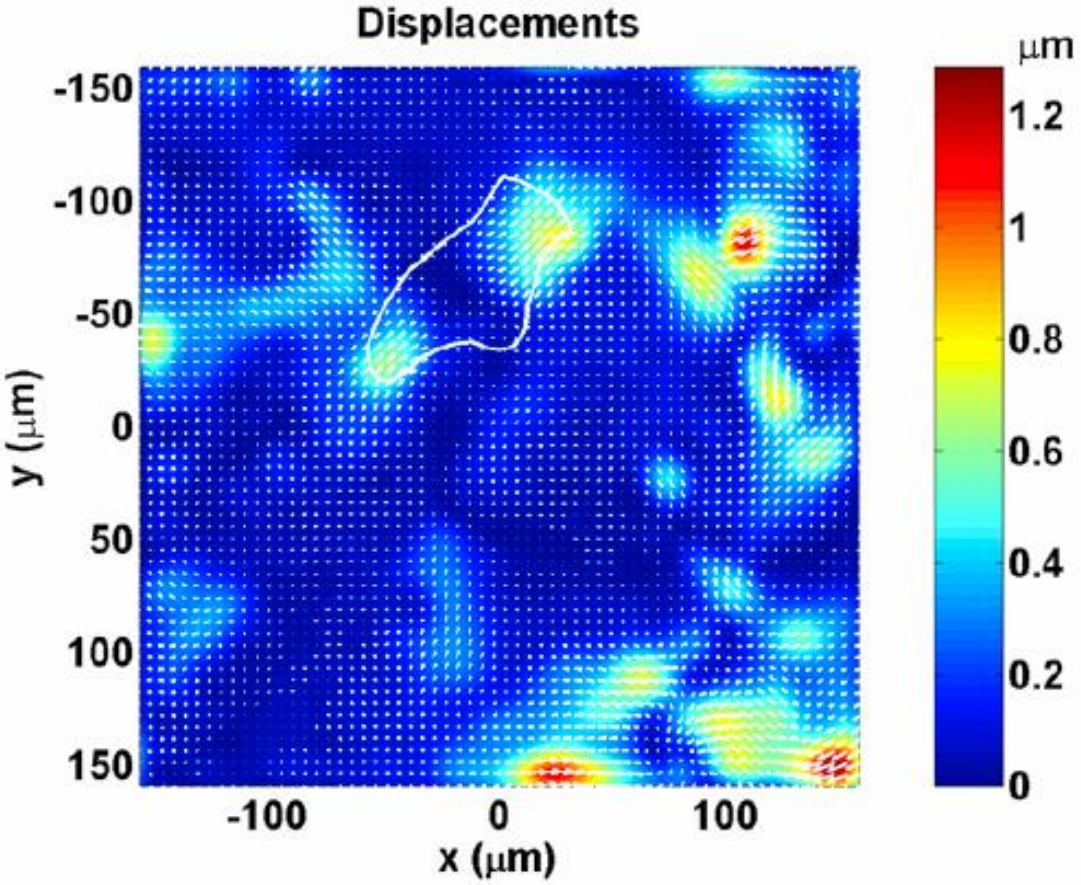
Prestress stiffens Filamin-Actin Networks



Traction force microscopy: Measures prestress in cell

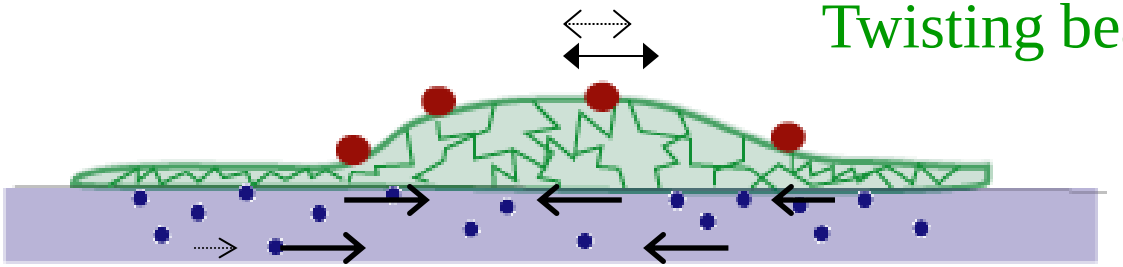


A7 Cells on 1kPa substrate, 24 hrs

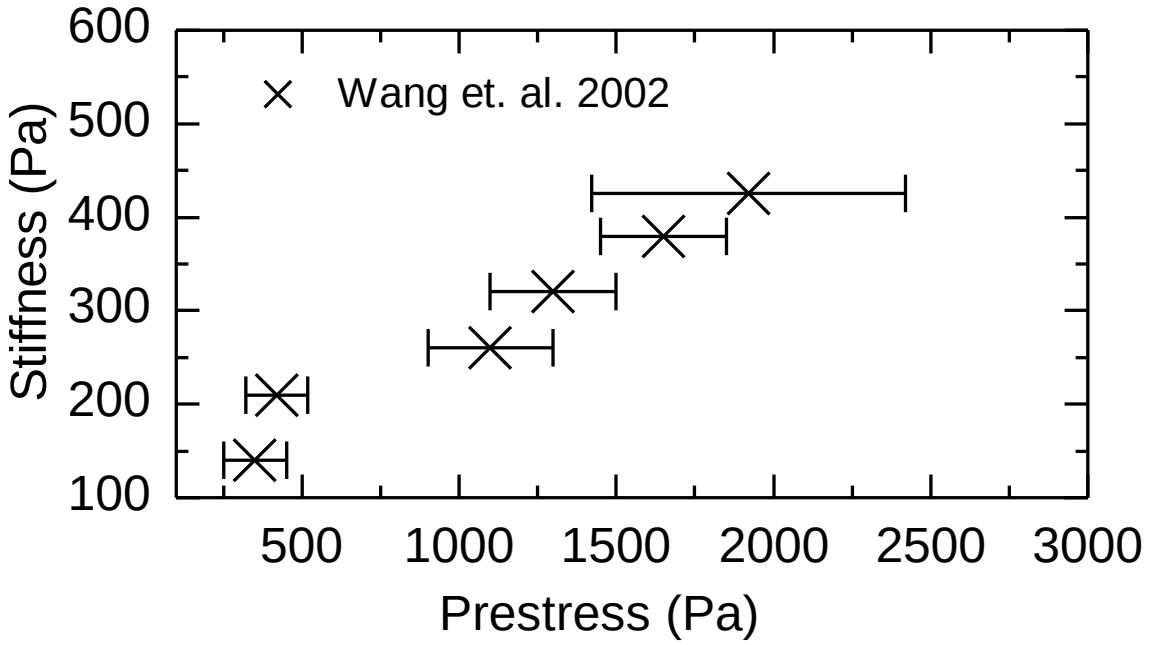


Cytoskeleton is pre-stressed

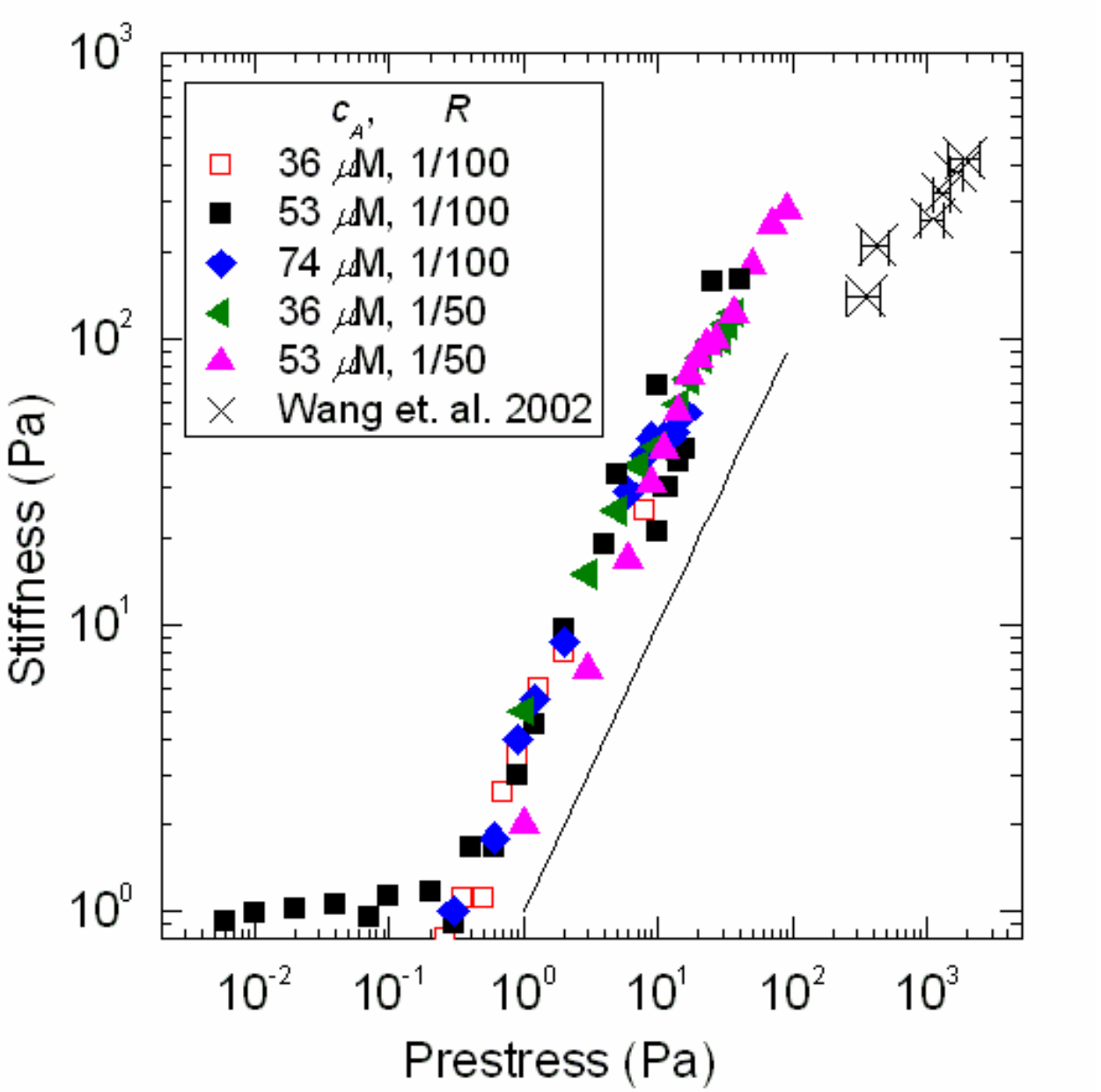
Twisting bead rheology



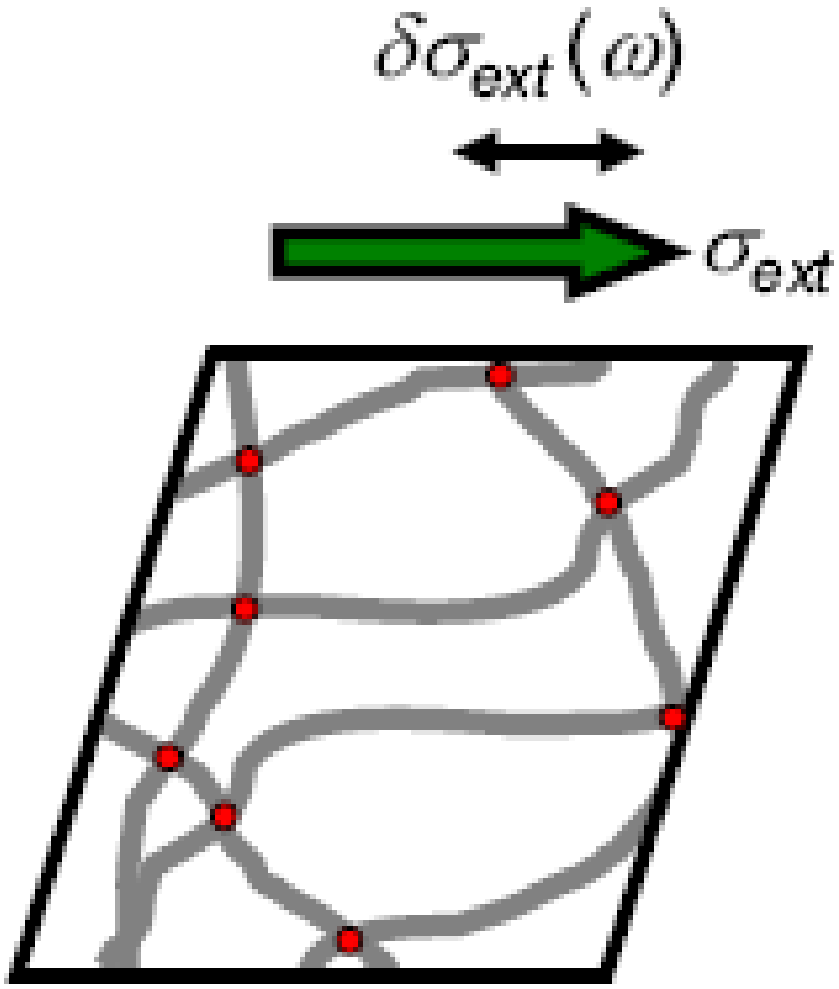
Traction Force Microscopy



Cell data agrees with filamin network

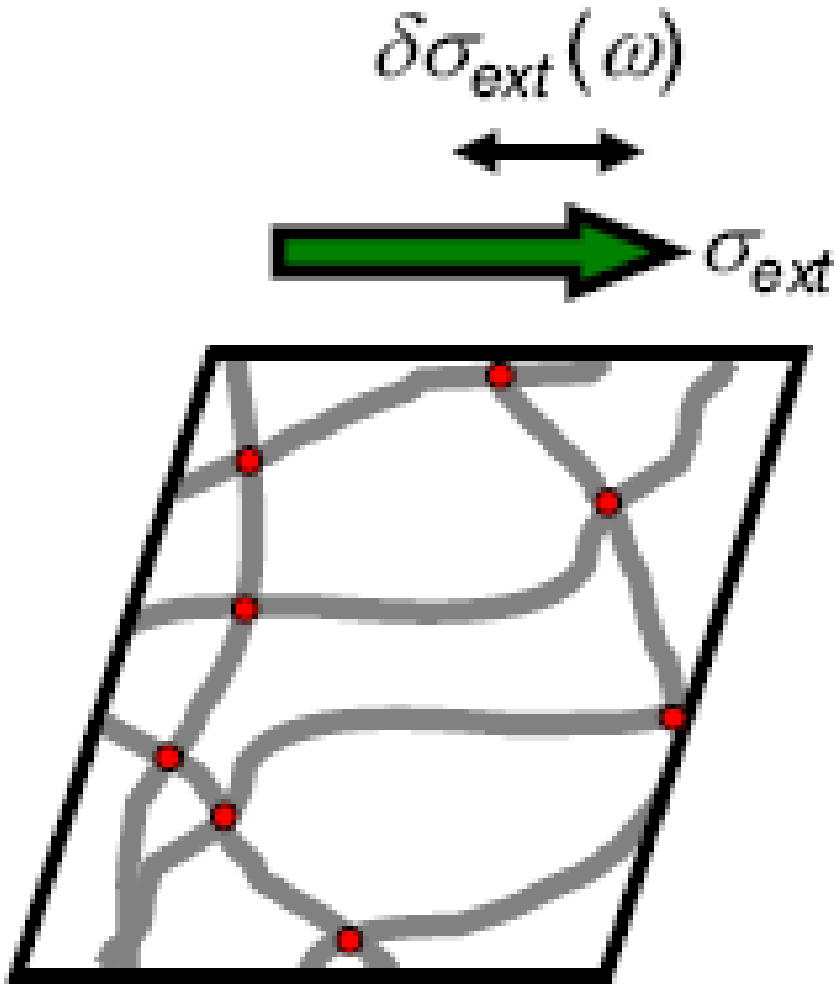


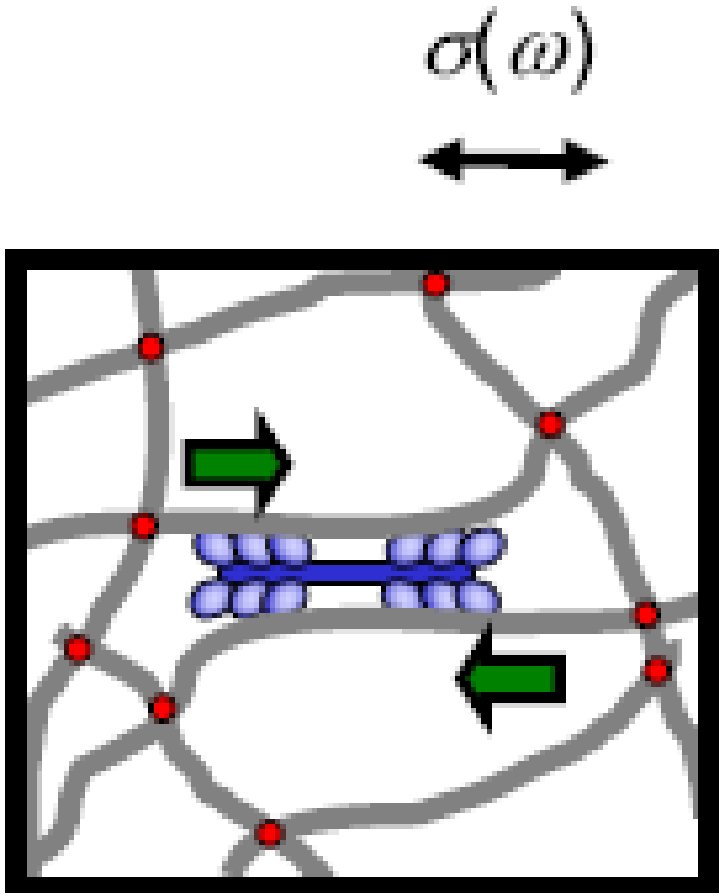
External strain puts tension on filaments



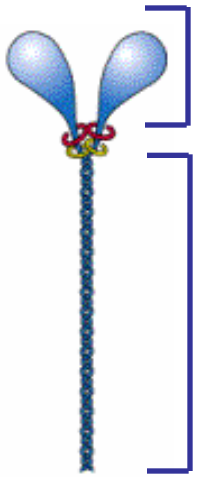
How to generate tension internally?

Can motors put internal tension on filaments??





Add motors: skeletal muscle myosin II

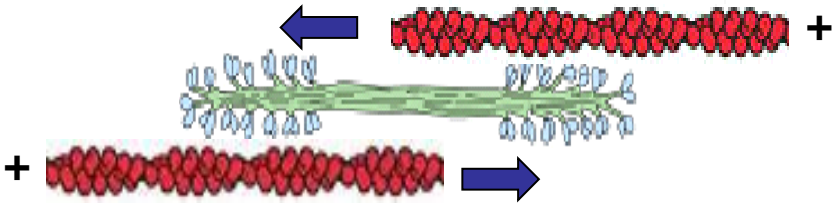


Non-processive heads:

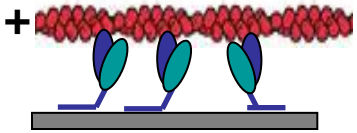
- ATP hydrolysis: 50 ms.
- Bound 1 ms, unbound 49 ms.

Tail:

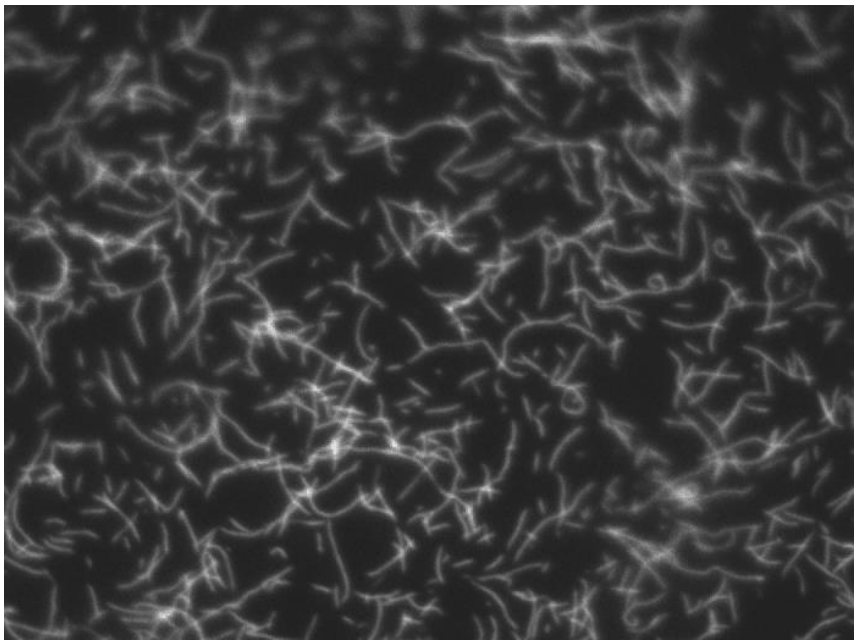
- Bipolar filament formation.
- Size: ionic strength, pH, T

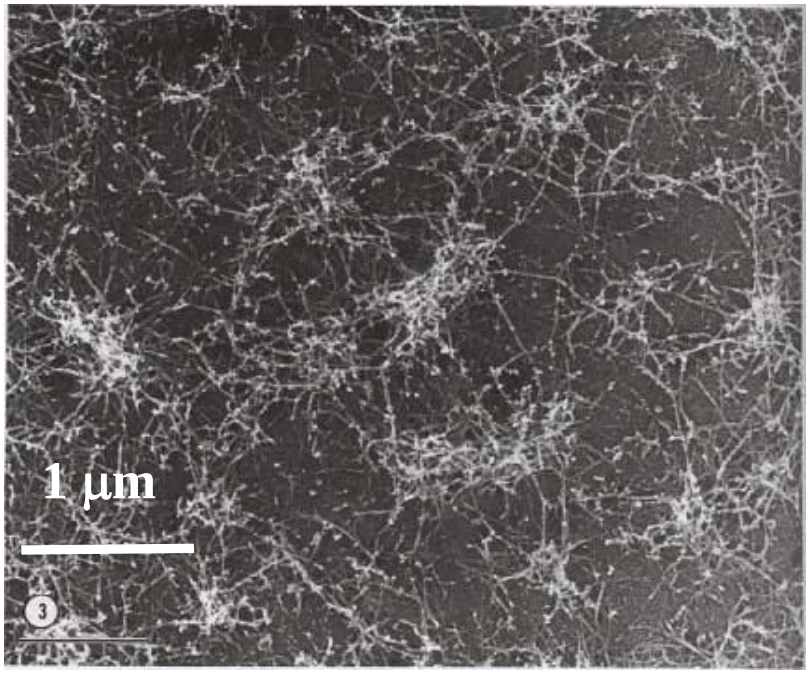


- 50 mM KCl, 5 mM MgATP.
- Length ~1 μm (~300 myosins).



Speed ~5 μm/s



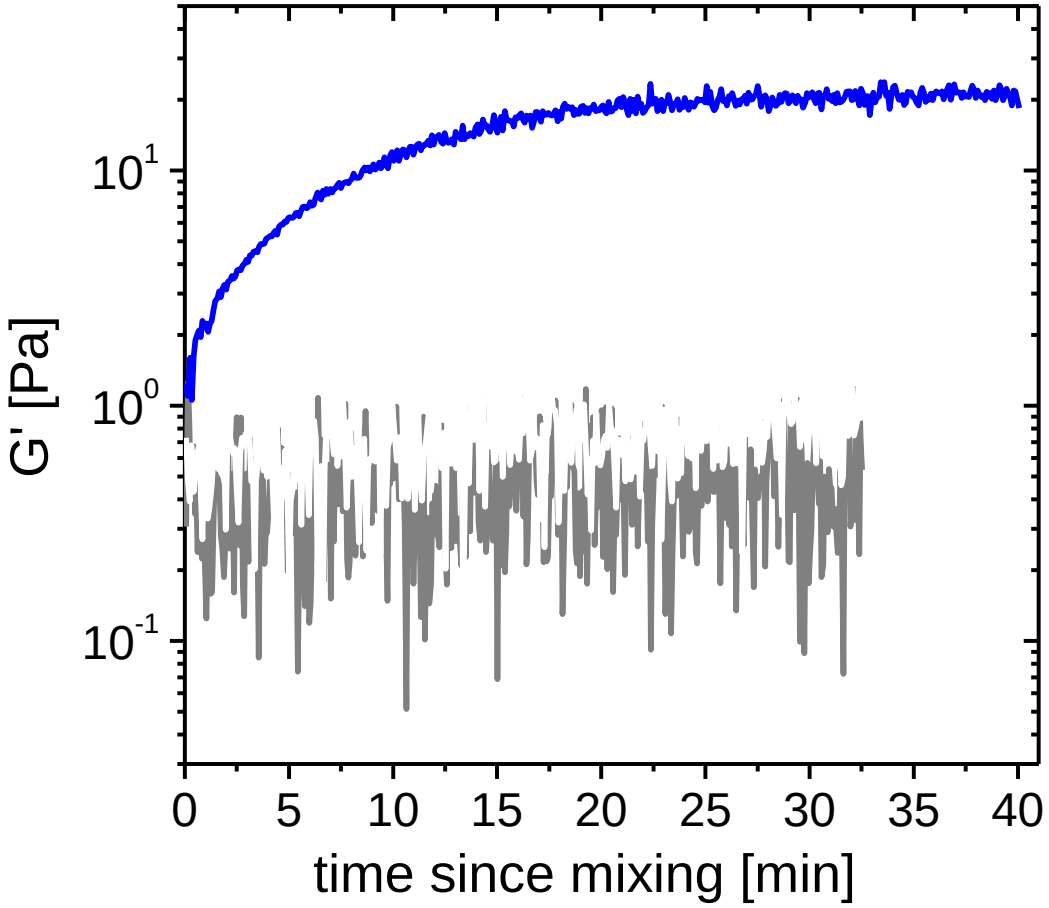


50 F-actin: 1 Filamin
Niederman, Amrein and Hartwig, JCB, **96** 1983

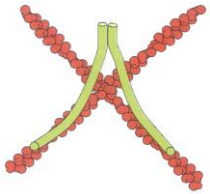
Stress build-up monitored with rheology

$\sigma(t) = \sigma_0 \sin(\omega t)$

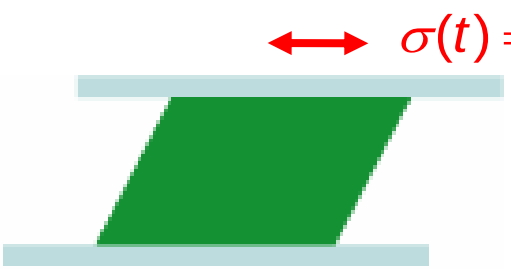
- Cone-plate 1°: gap 30 – 80 µm.
- Probe with small strain <1%, 1 Hz.



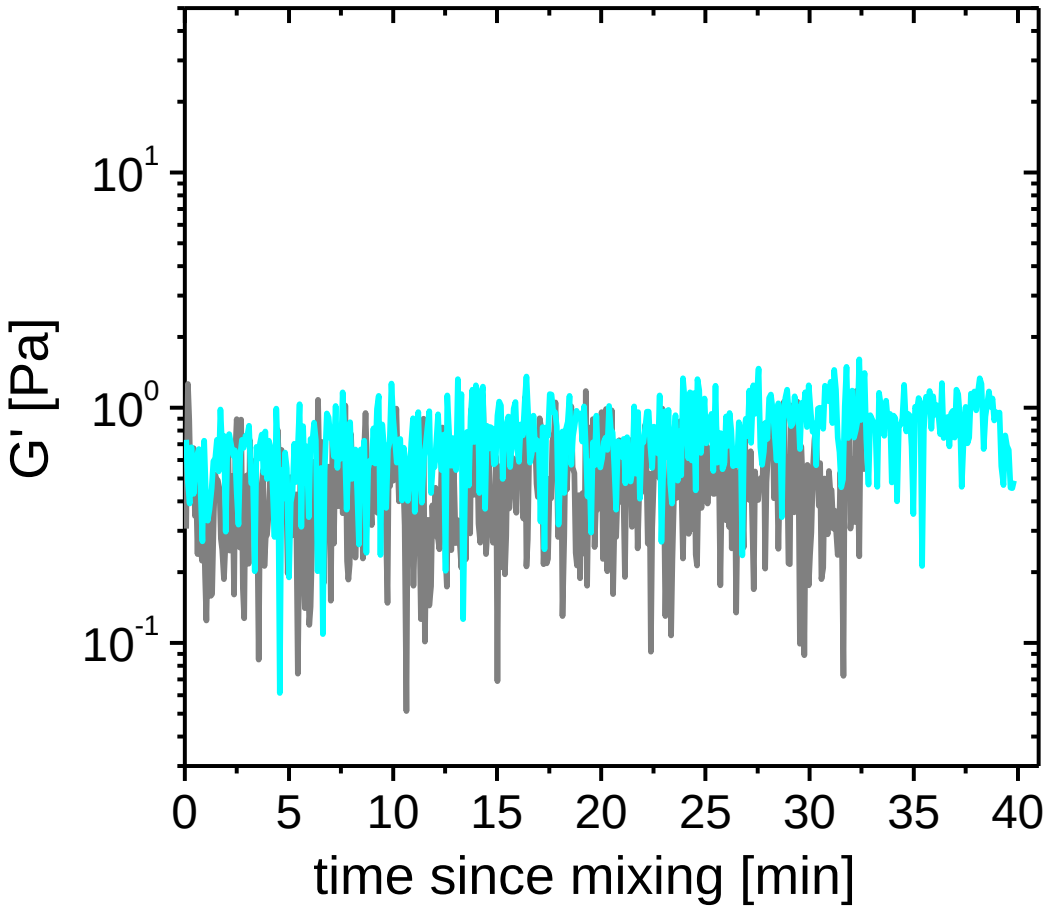
- Actin + myosin (1/50)
- Add filamin (1/100)



Stress build-up monitored with rheology

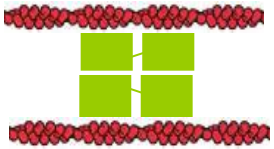


- Cone-plate 1°: gap 30 – 80 μm.
- Probe with small strain <1%, 1 Hz.

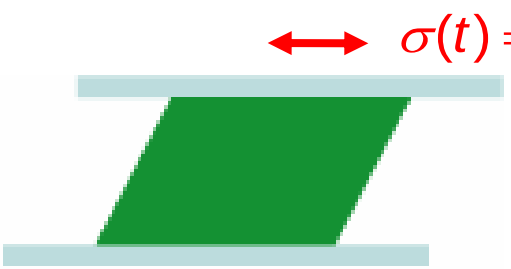


➢ Actin + myosin (1/50)

➢ Add scruin (1/100)



Stress build-up monitored with rheology



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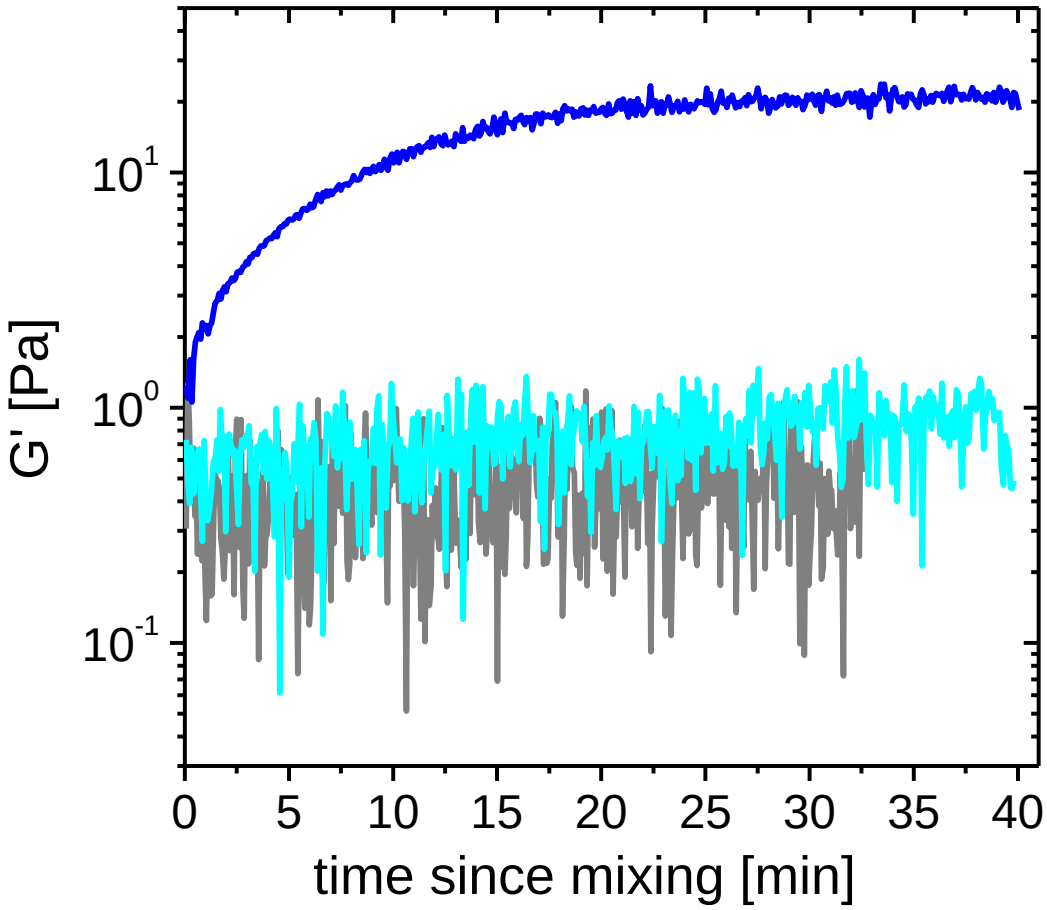
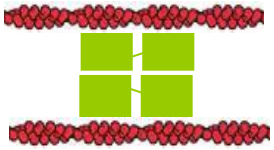
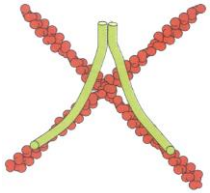


➢ Actin + myosin (1/50)

➢ Add scruin (1/100)



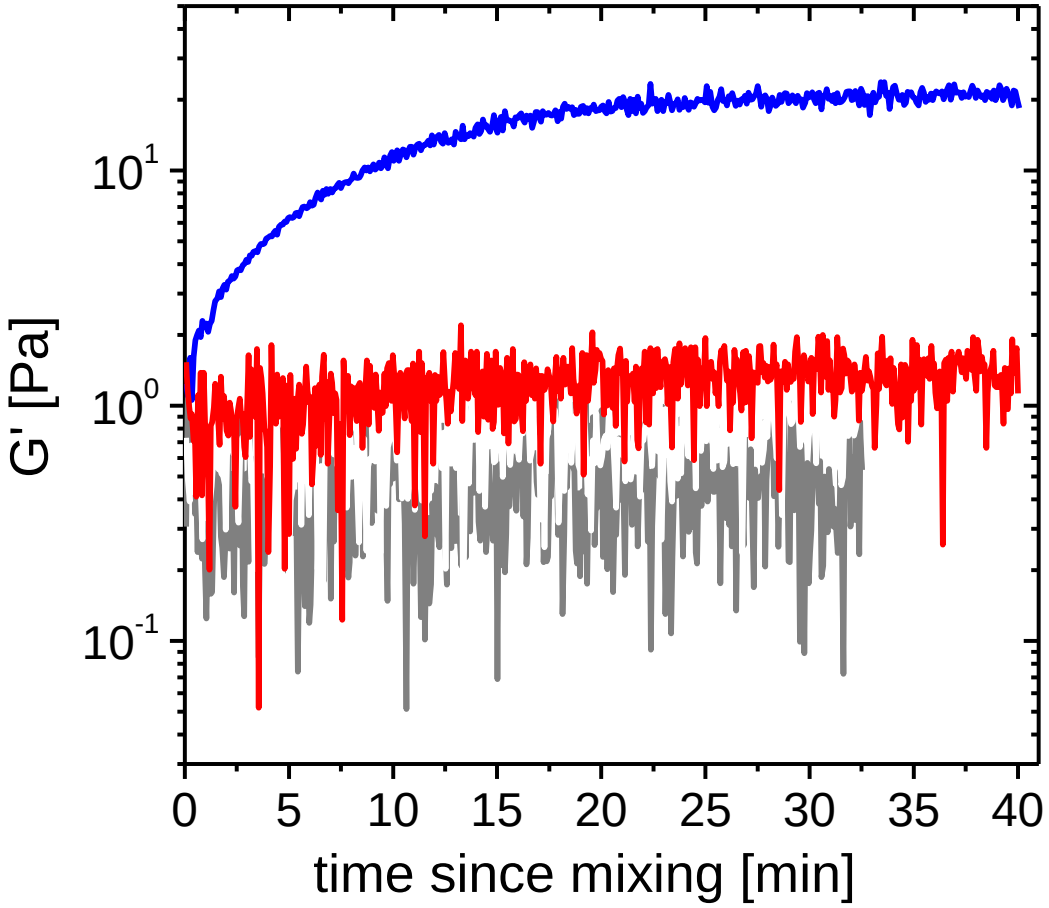
➢ Add filamin (1/100)



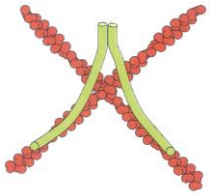
Stress build-up monitored with rheology

$\sigma(t) = \sigma_0 \sin(\omega t)$

- Cone-plate 1°: gap 30 – 80 µm.
- Probe with small strain <1%, 1 Hz.

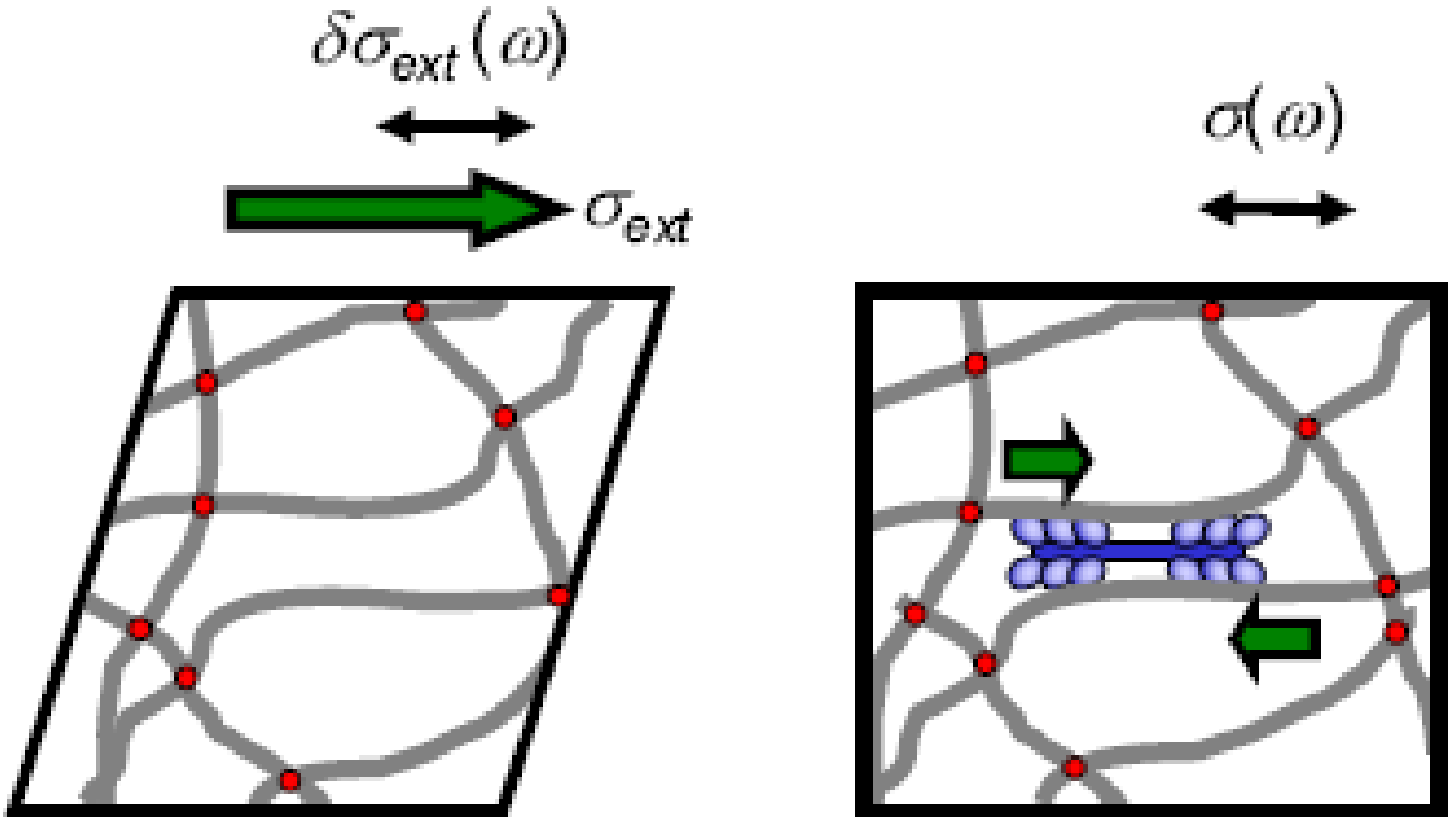


- Actin + myosin (1/50)
- Add filamin (1/100)

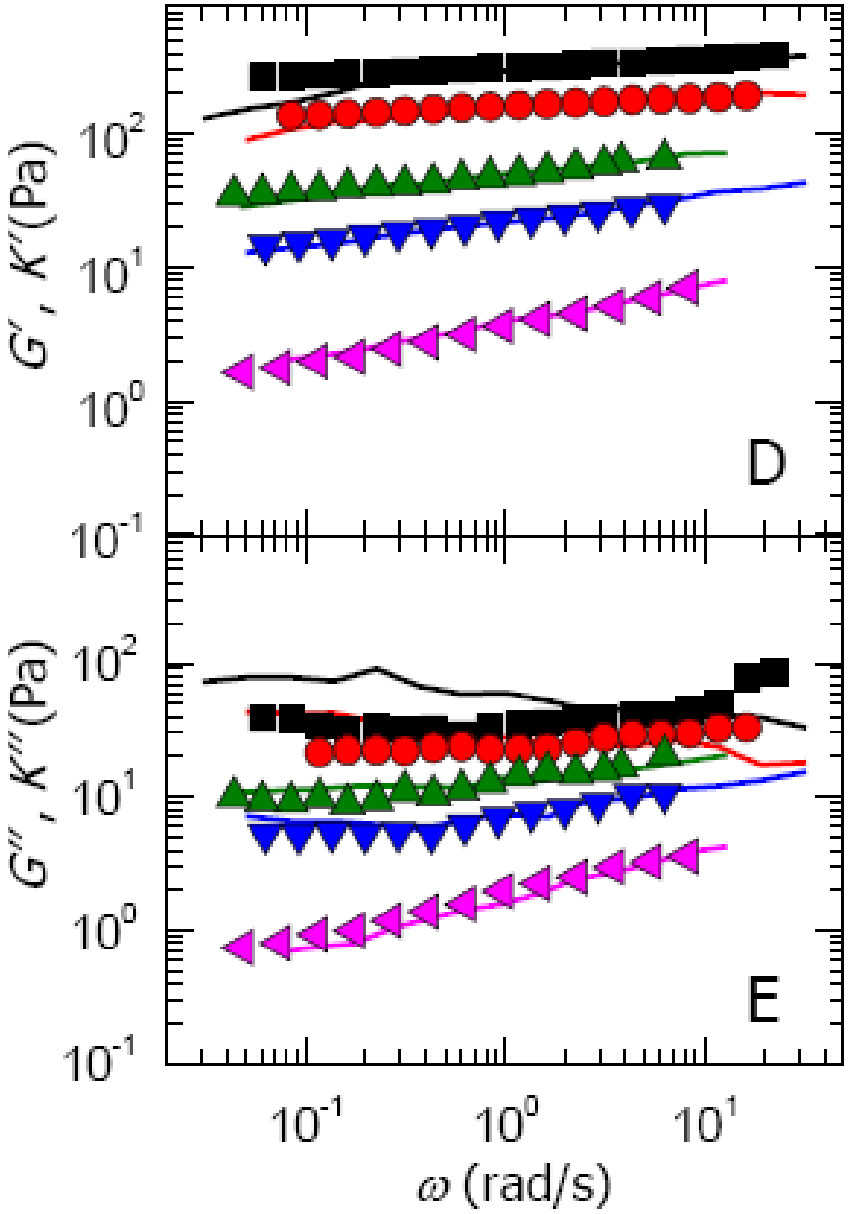


- Add blebbistatin (inhibit myosin ATPase)

Calibrate internal tension



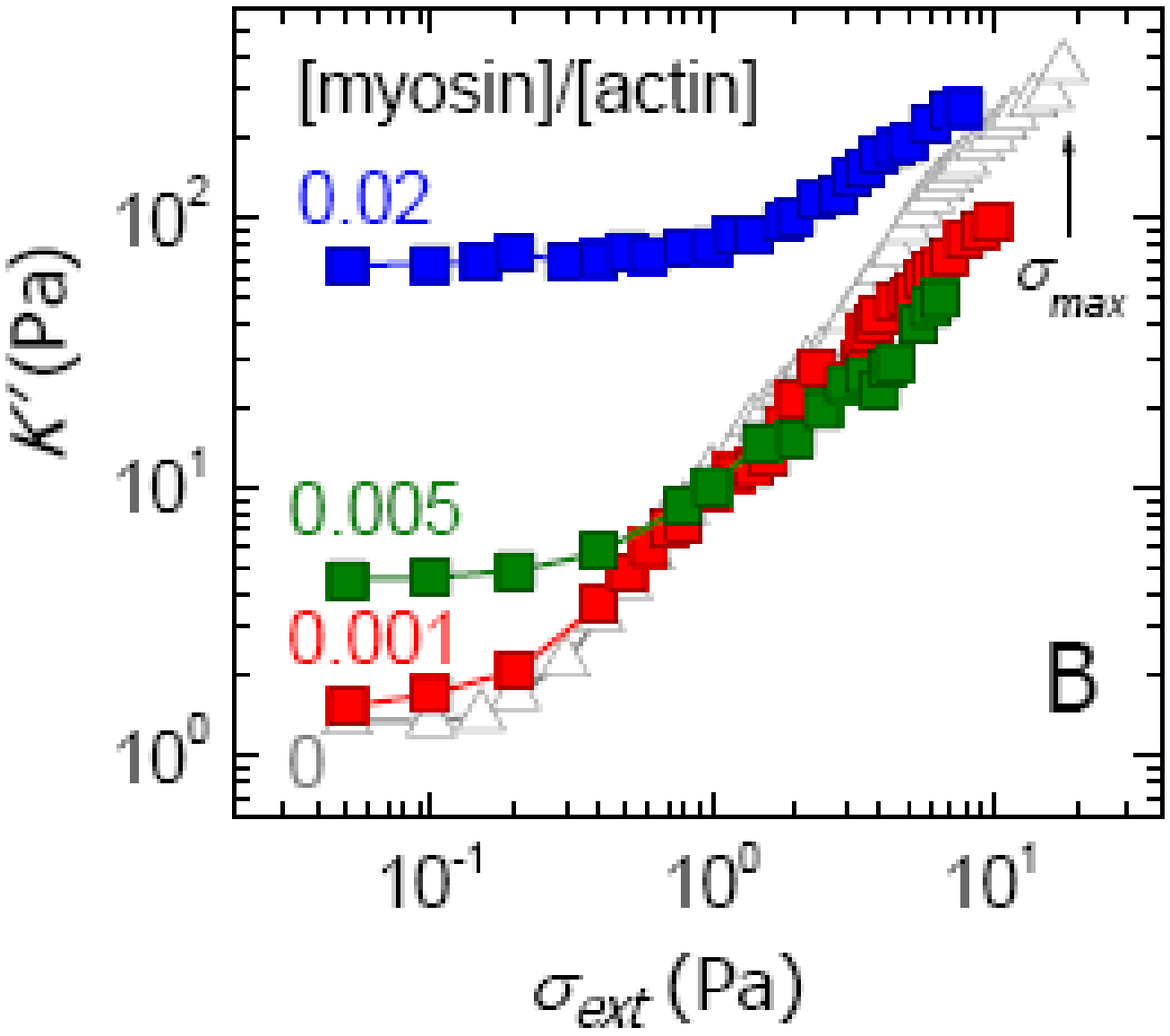
Internal stress identical to external stress



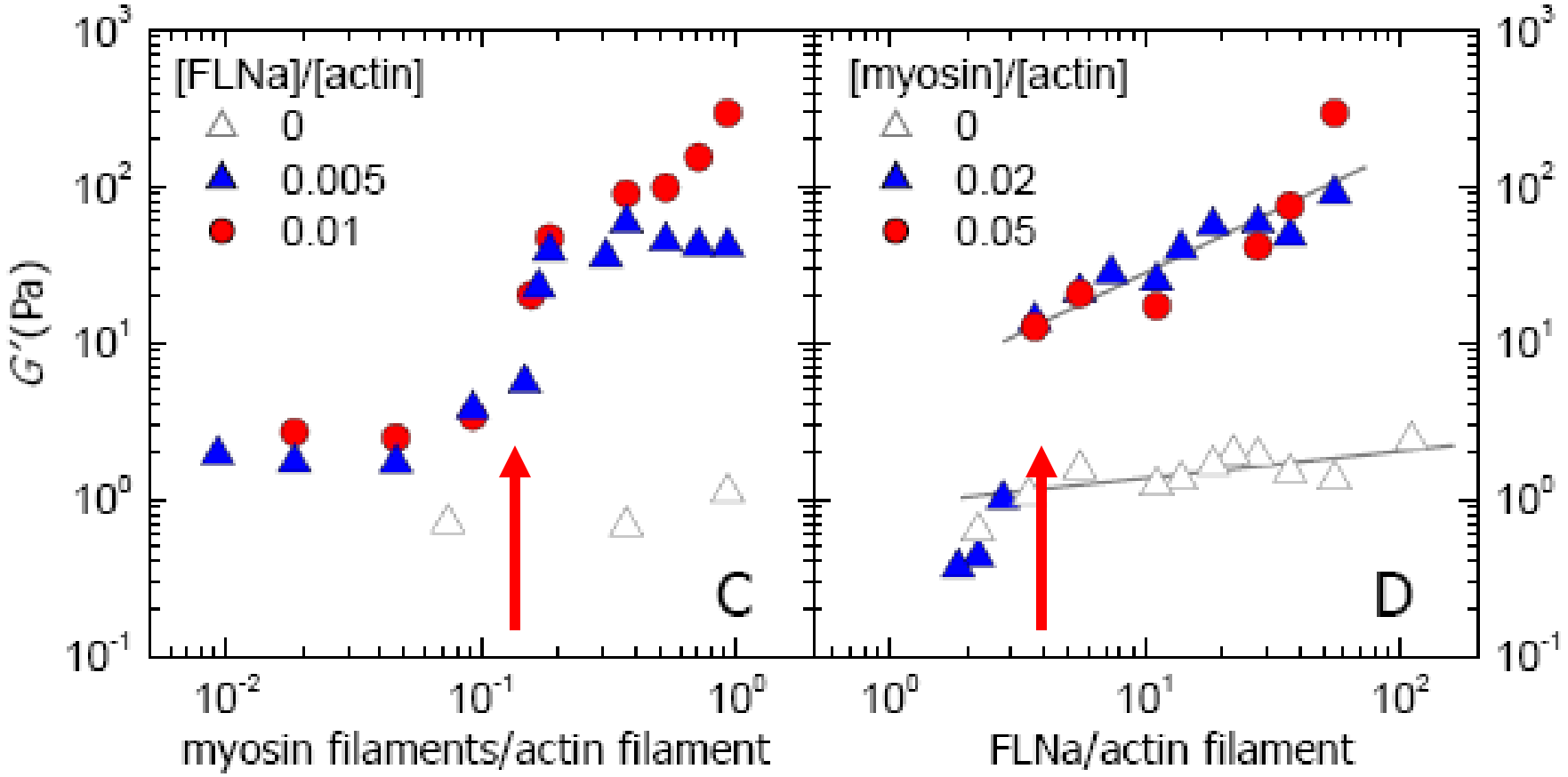
Calibration

But:
Anisotropic
vs
Isotropic

Internal stress identical to external stress

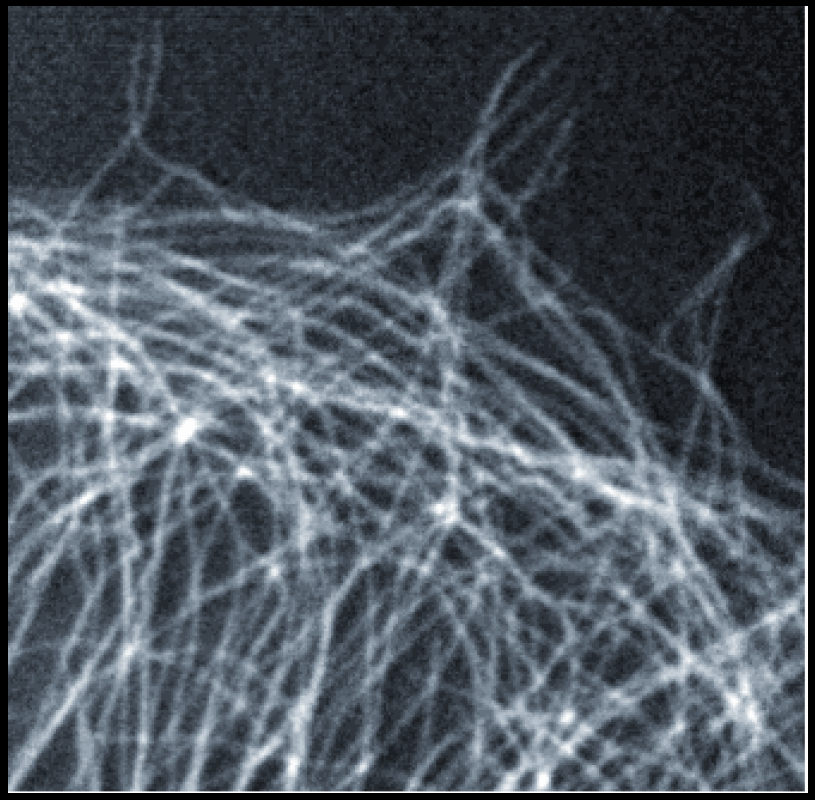


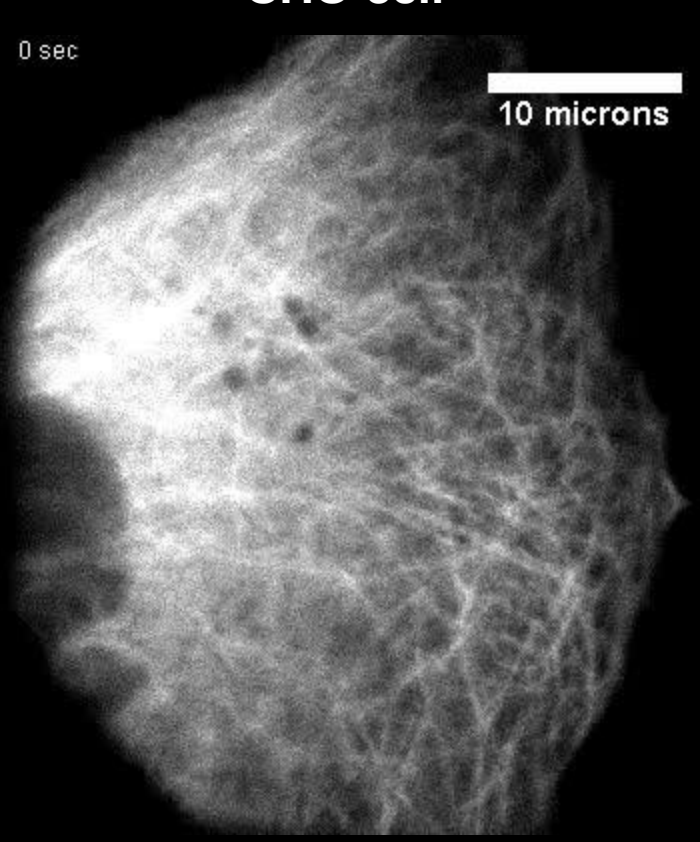
Depends on actin filaments



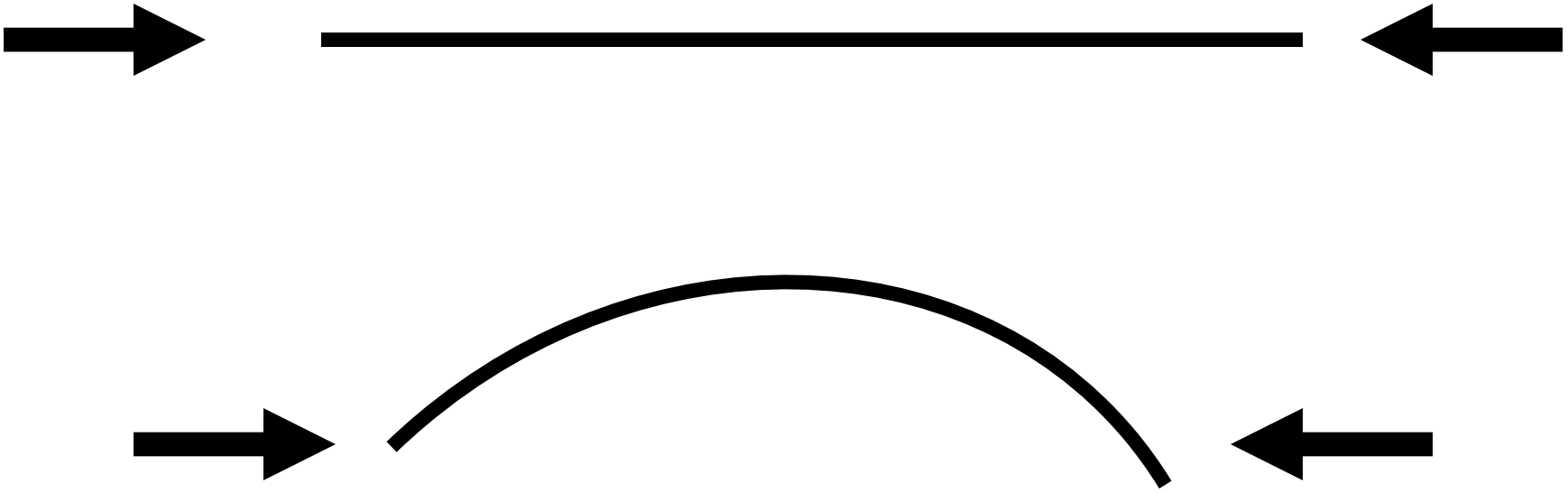
Sharp onset → minimum number required

Microtubule fluctuations





Compressive stresses on microtubules

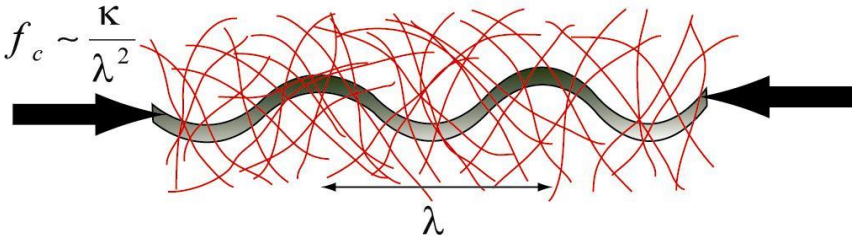


Euler buckling

Compressive stresses on microtubules

Minimize energy by buckling at a certain wavelength

$$f_c \sim \frac{\kappa}{\lambda^2}$$

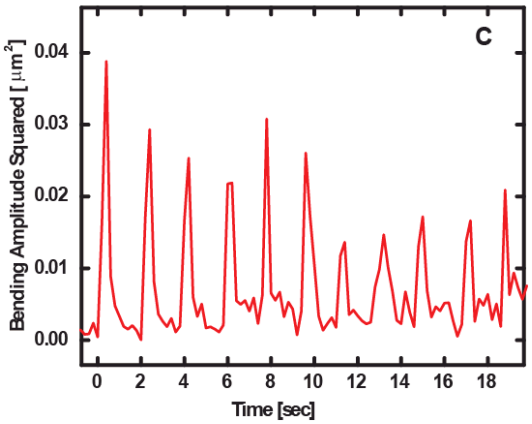


$$\lambda \sim \left(\frac{\kappa}{G}\right)^{1/4}$$

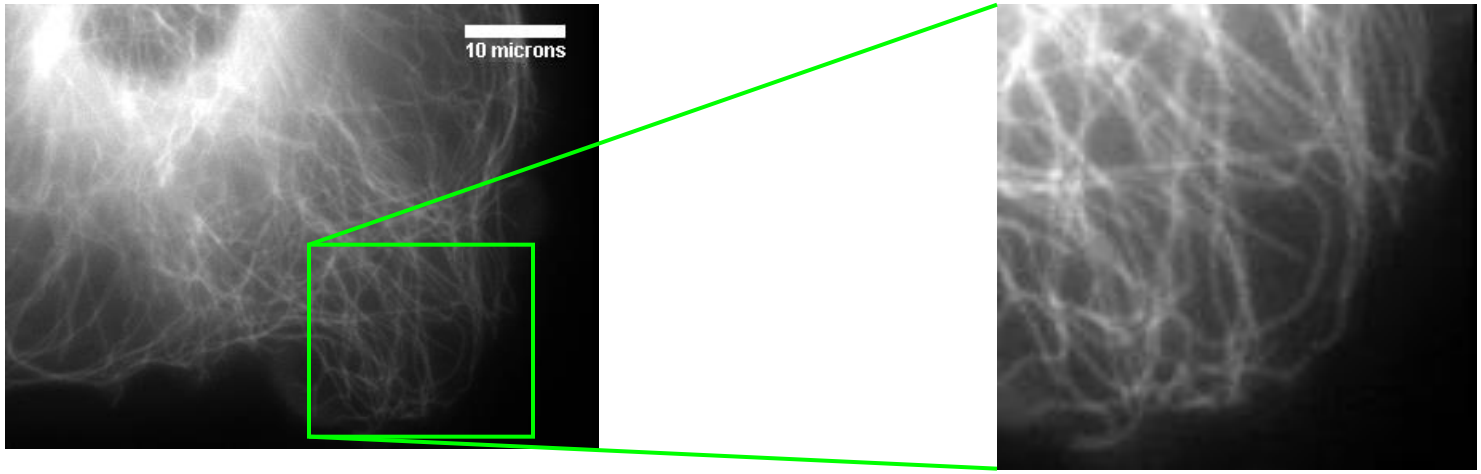
$\kappa =$ MT bending rigidity

$G =$ Elastic modulus of surrounding material

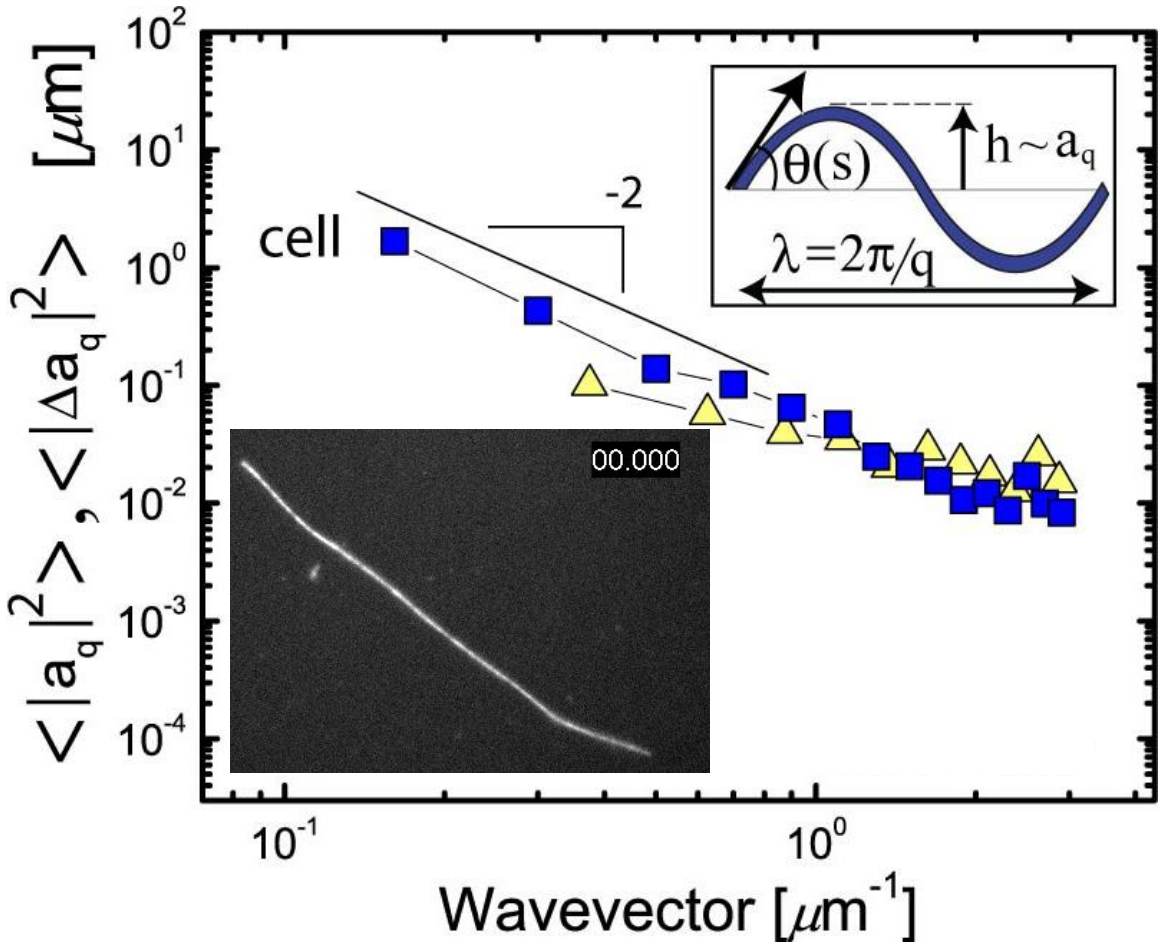
Periodic MT Buckling in heart cells



Buckling from exogenous compressive forces

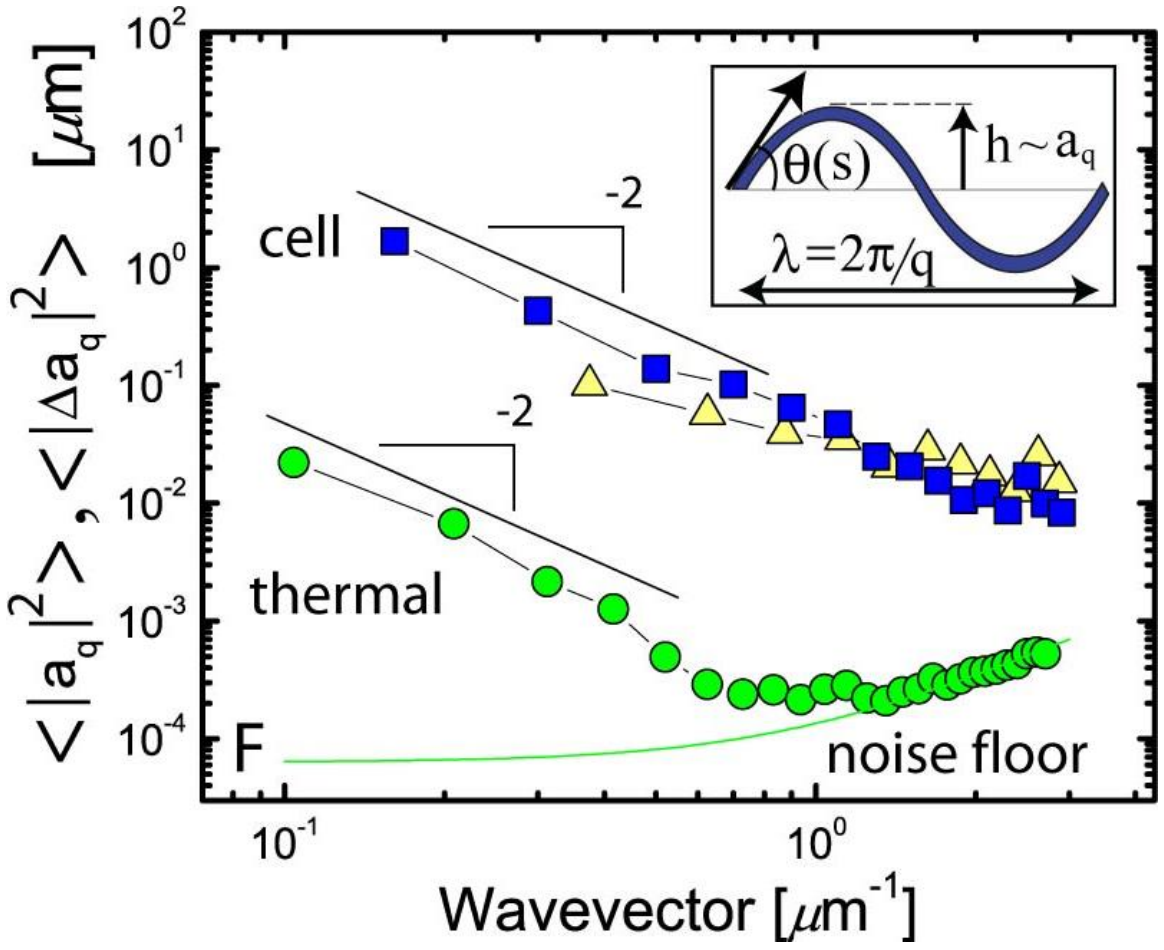


Fourier Mode Analysis - Ensemble



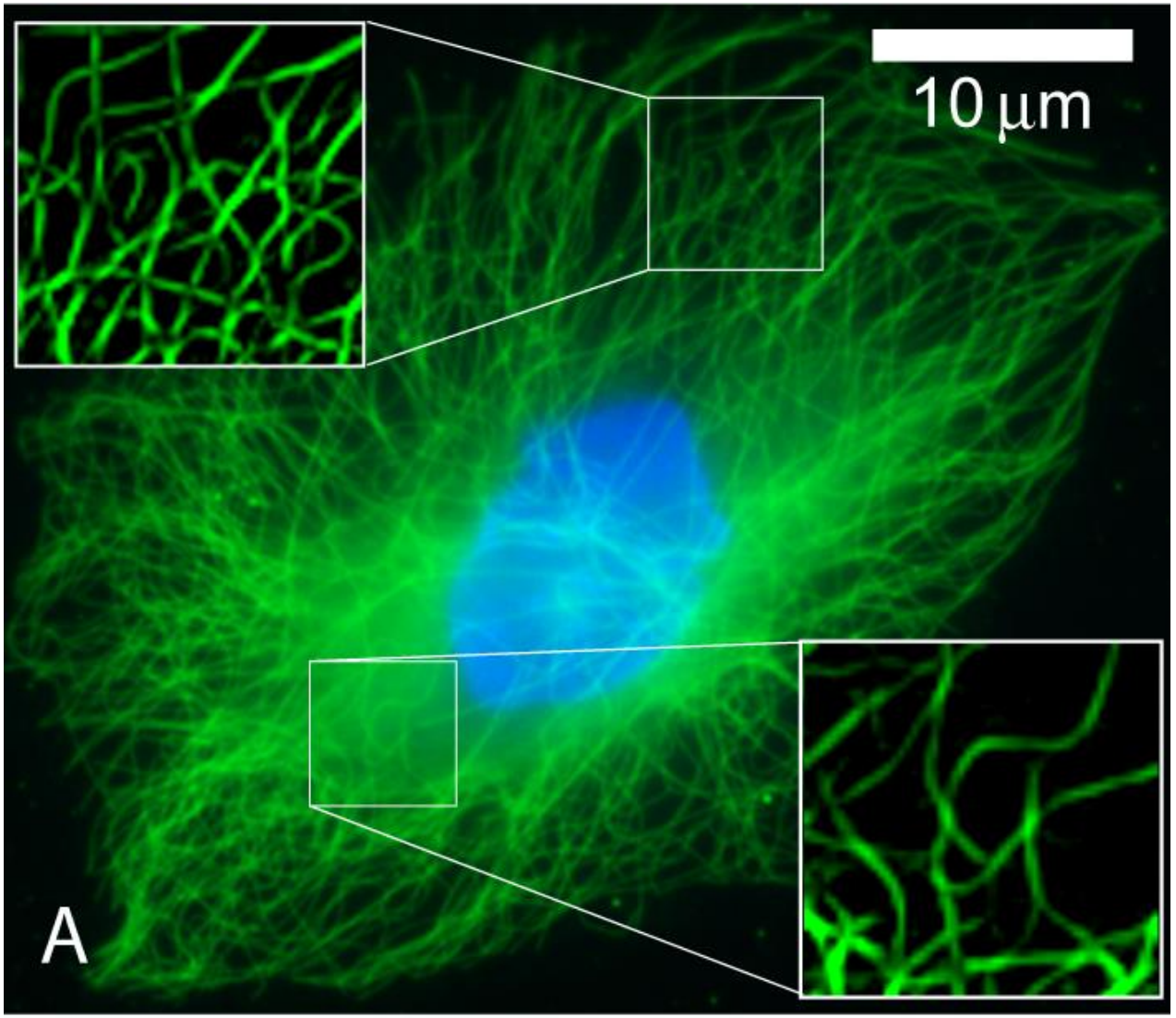
- Thermal-like spectrum

Fourier Mode Analysis - Ensemble



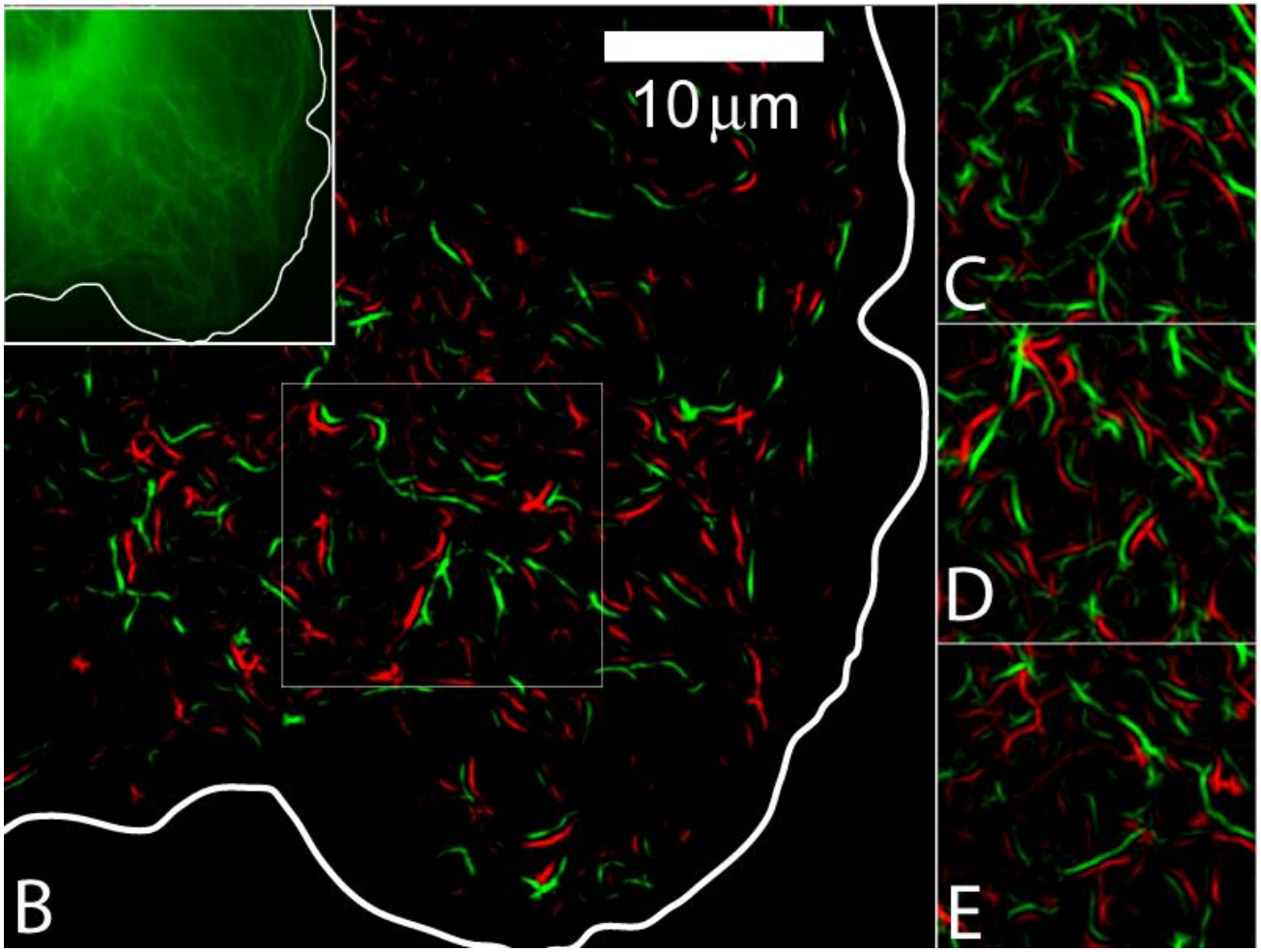
- Thermal-like spectrum
- Much larger amplitude

Microtubules can be highly bent



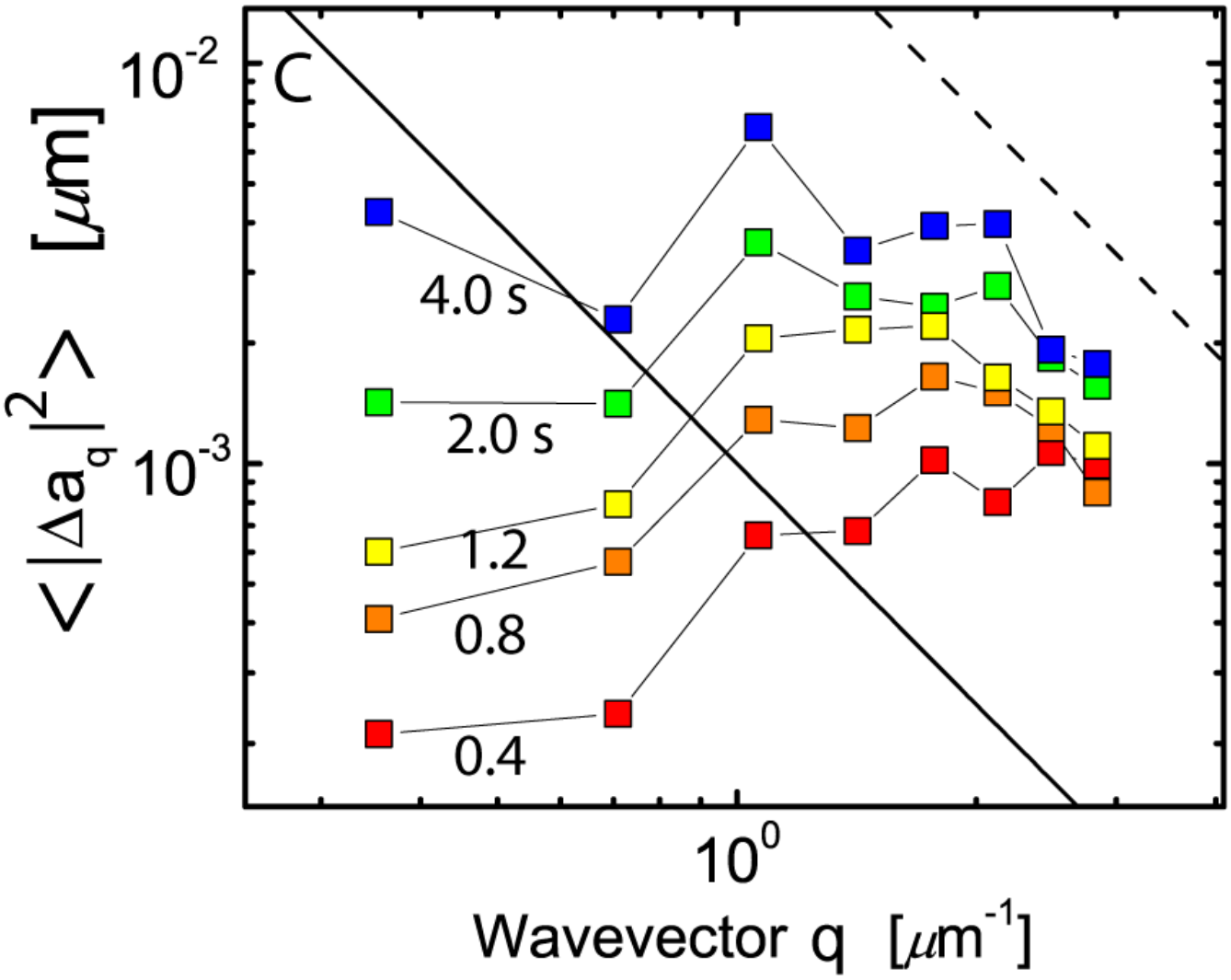
Fixed CHO cell

Fluctuations are short wavelength



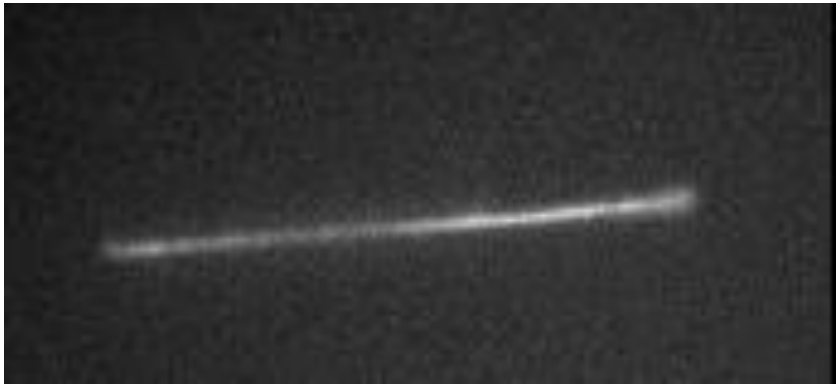
- GFP-tubulin transfected Cos7 cell
- Time difference – 1.6 seconds
- New position – red; earlier position – green

Fluctuation spectrum of single microtubule



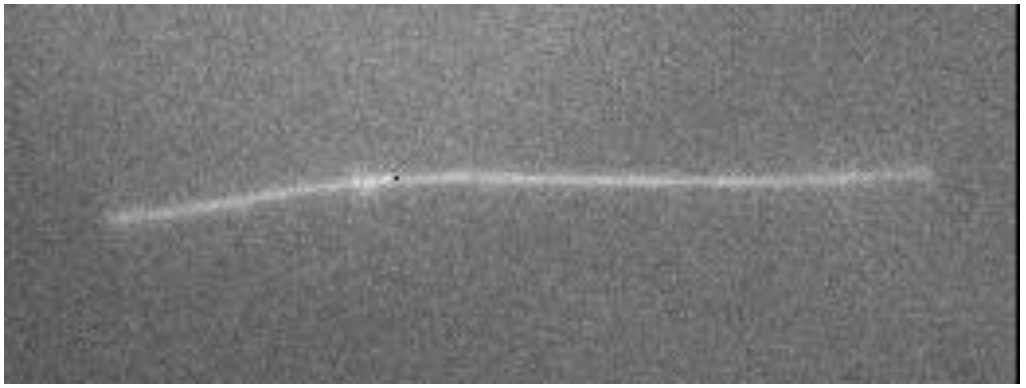
Microtubules confined in actomyosin networks

Act 24 μM, 1:50 myo, L= 19 μm



140 frames, 42 s total

Act 24 μM, 1:100 myo, L=32 μm

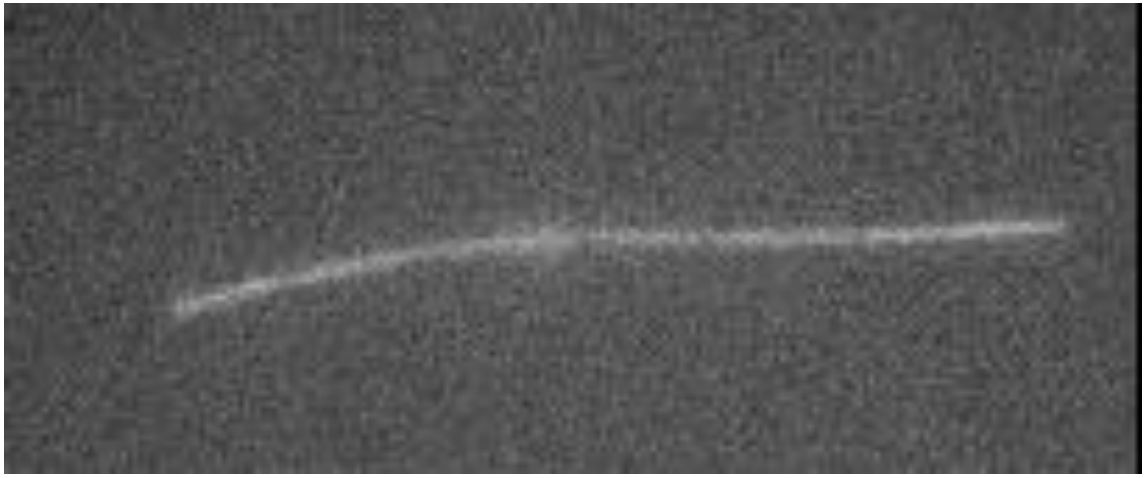


906 frames, 4½ min total

Act 24 μM, 1:50 myo, L=19 μm

1200 frames, 6 min total

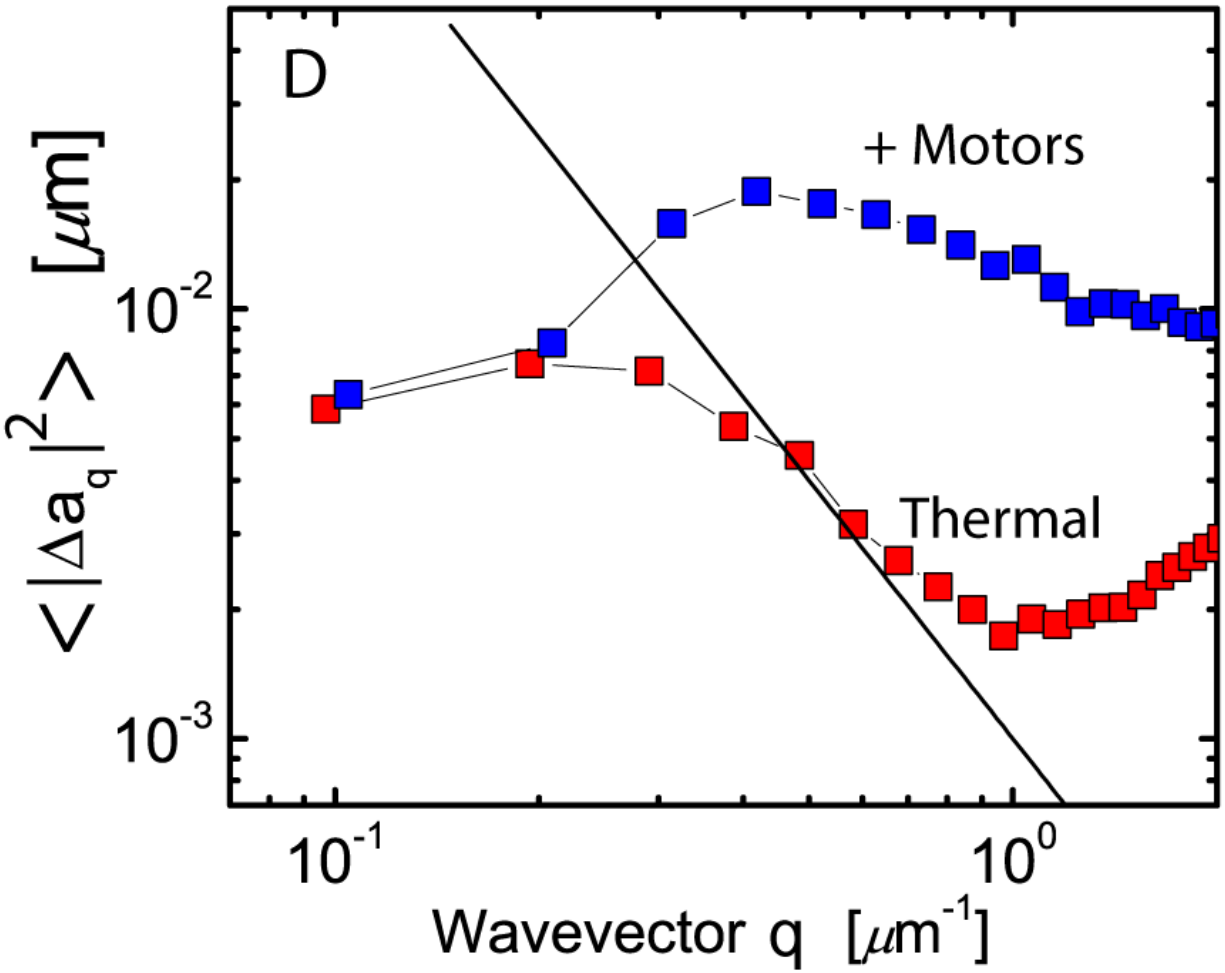
Act 24 μM, 1:100 myo, L=43 μm



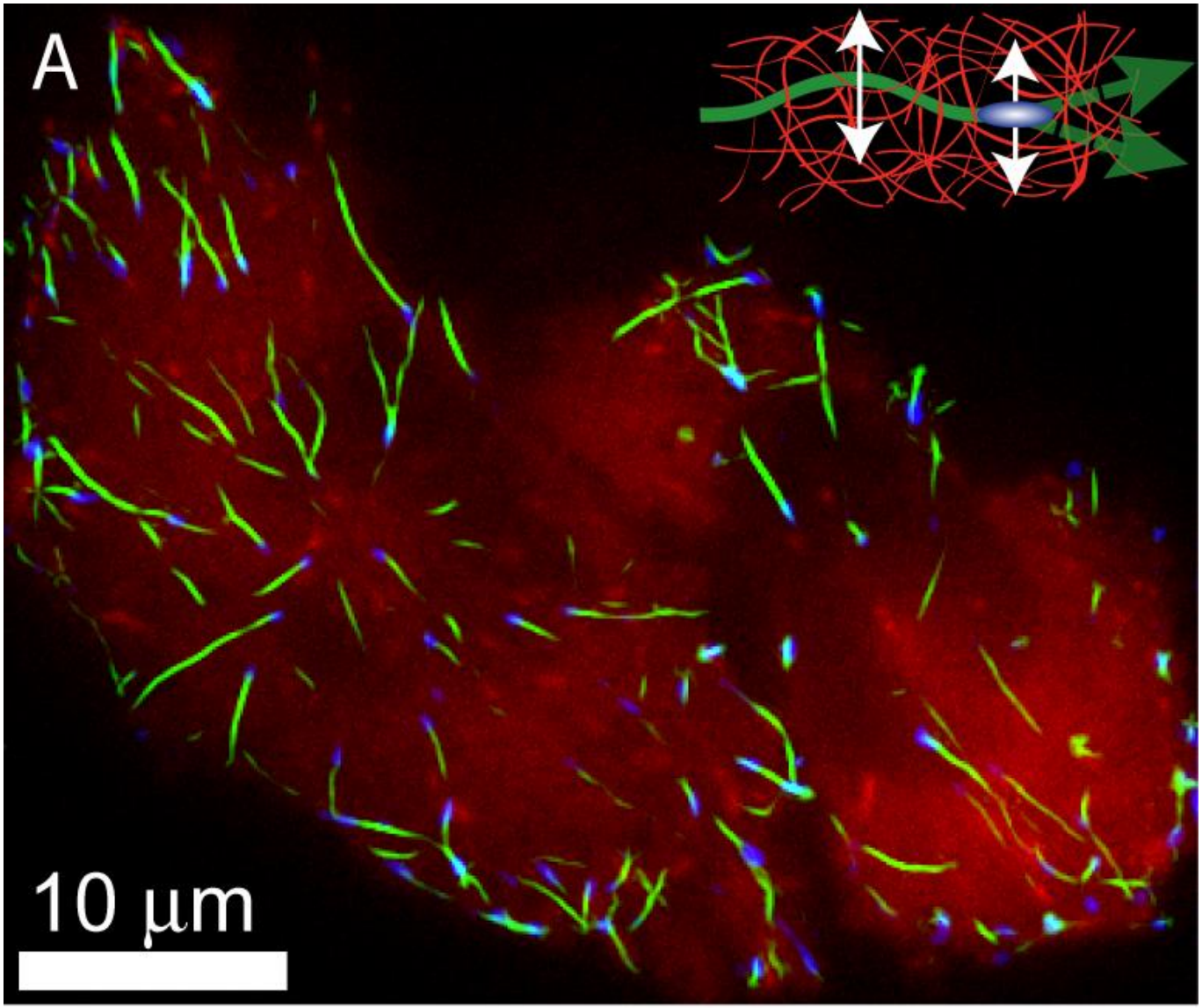
300 frames, 1½ min total

All movies: 0.3 s between frames, 2x sped up.

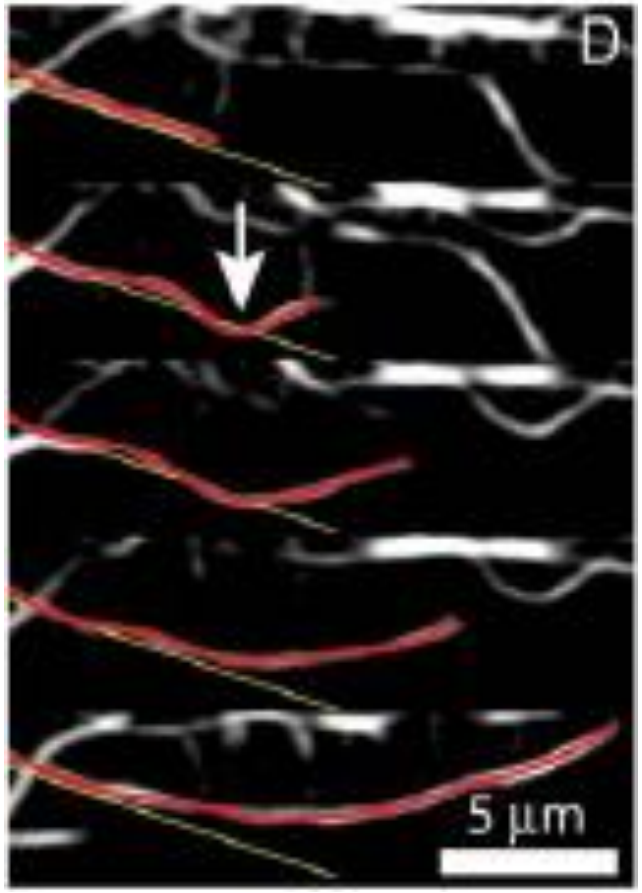
Fourier Mode Analysis: Single Microtubule *in vitro*

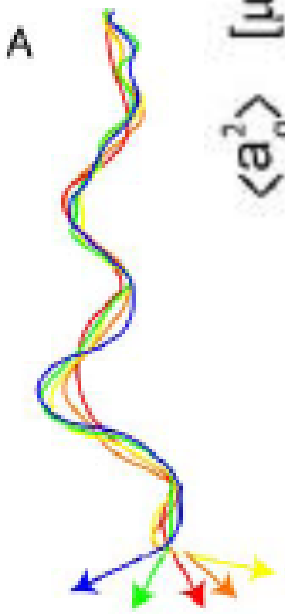


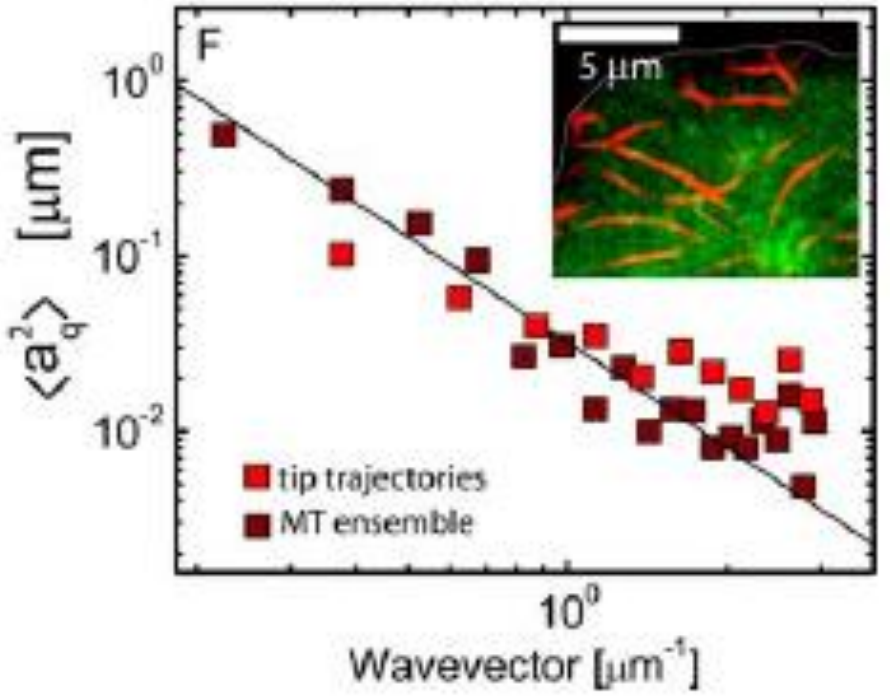
Tip growth of microtubules



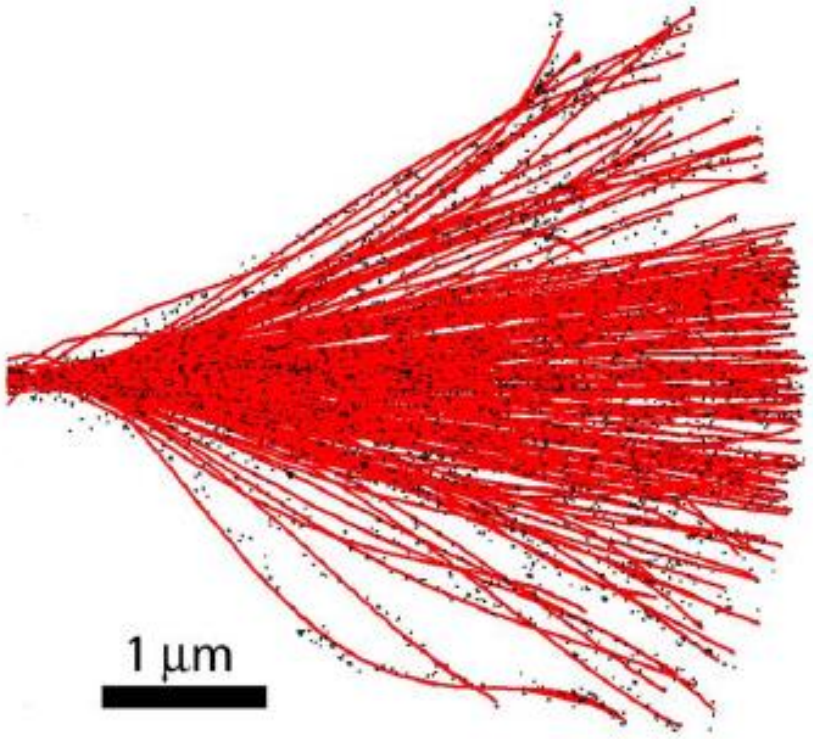
Fluctuations of Tip Growth





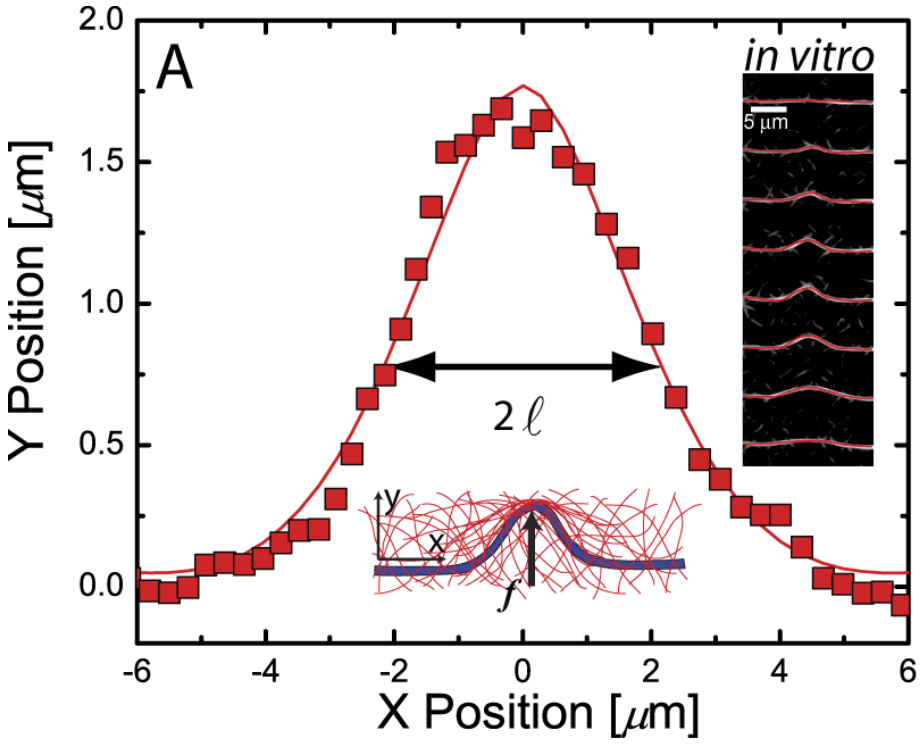


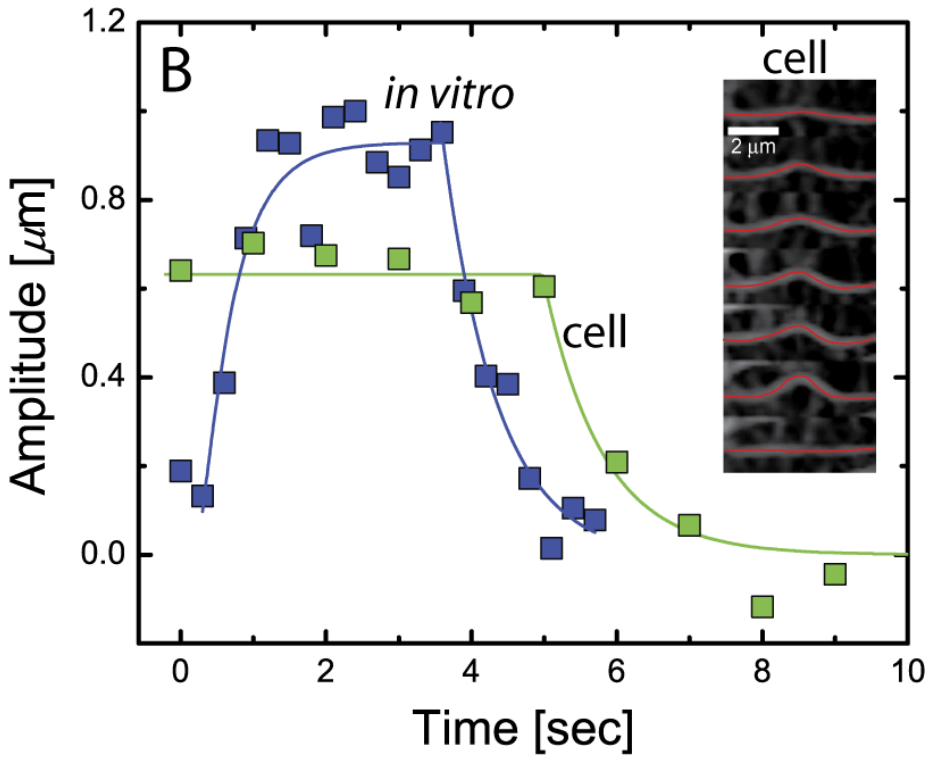
Tip Growth: Persistent Random Walk



- Larger polymerization forces
- Improved "search and capture"

Forces on microtubules

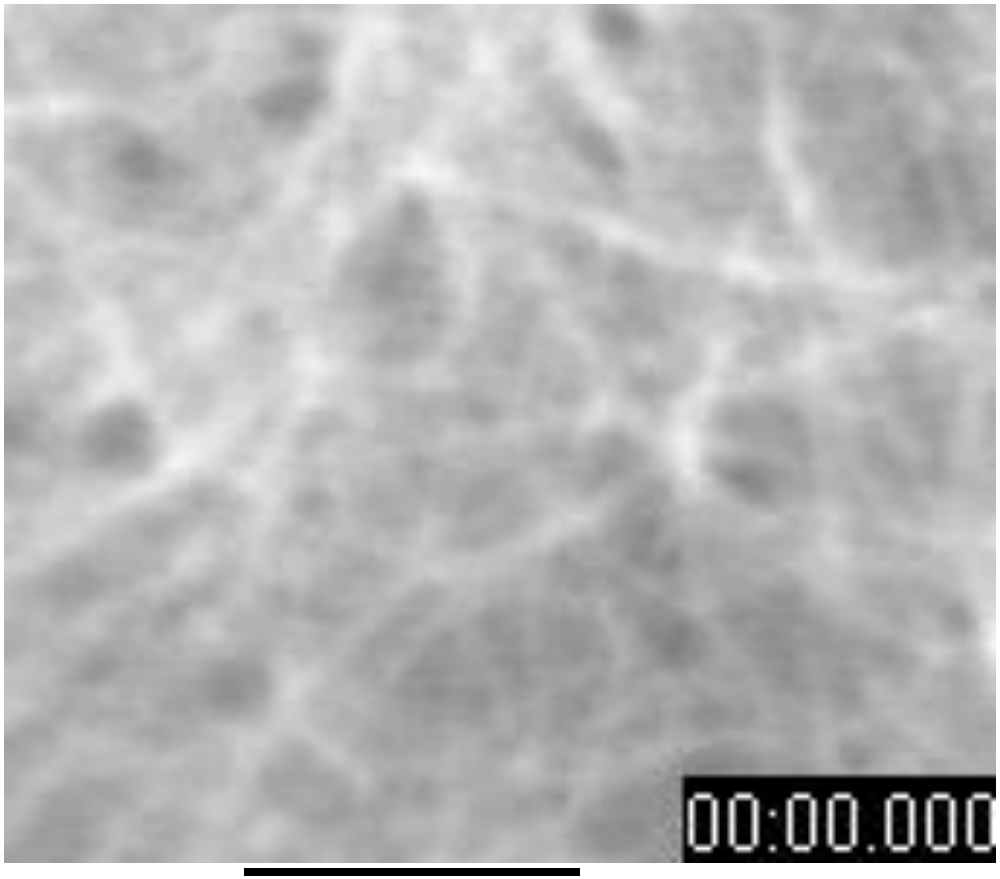




Point force

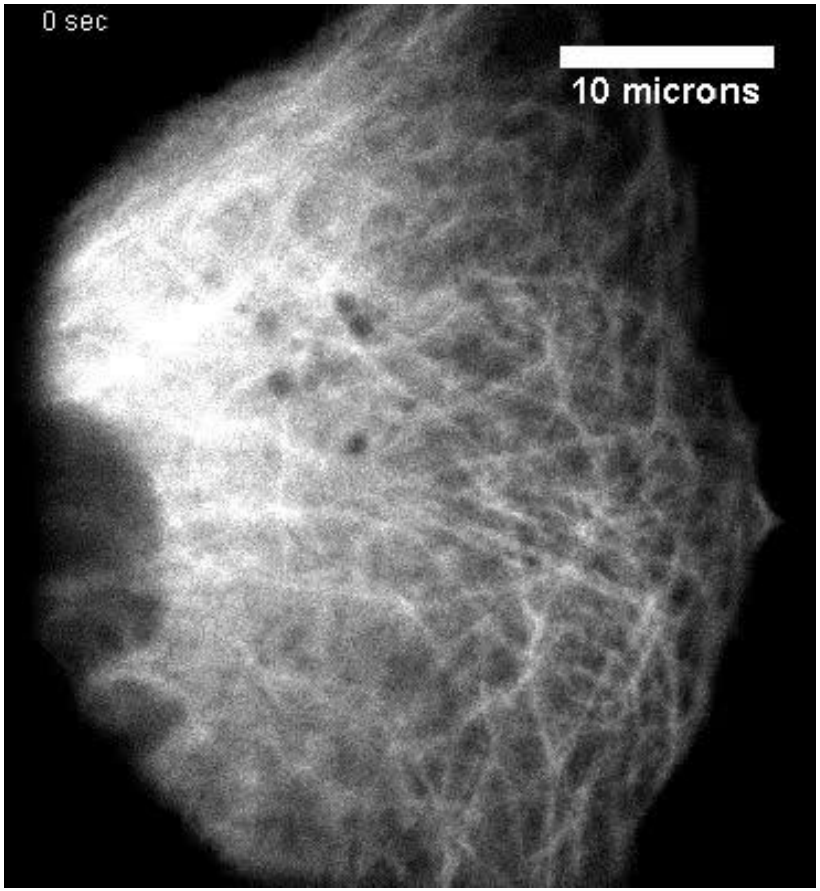
Exponential time dependence

Fluctuating motion within cells



5 μm

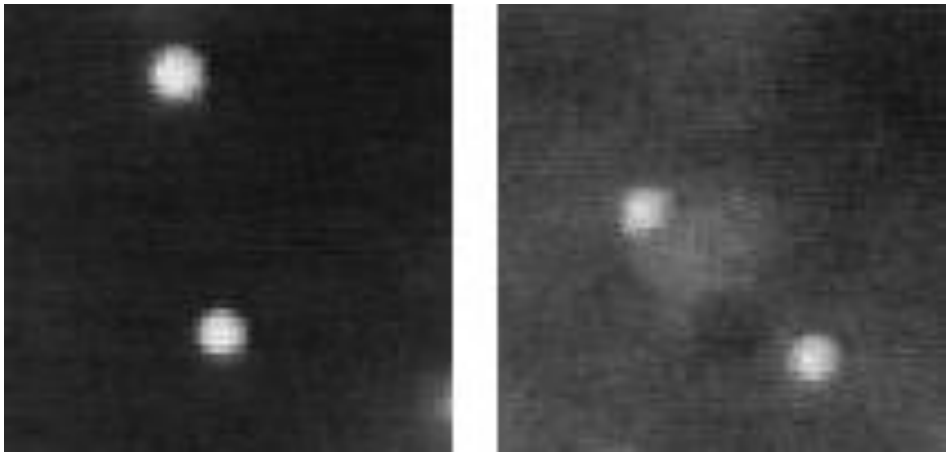
Endogenous granules



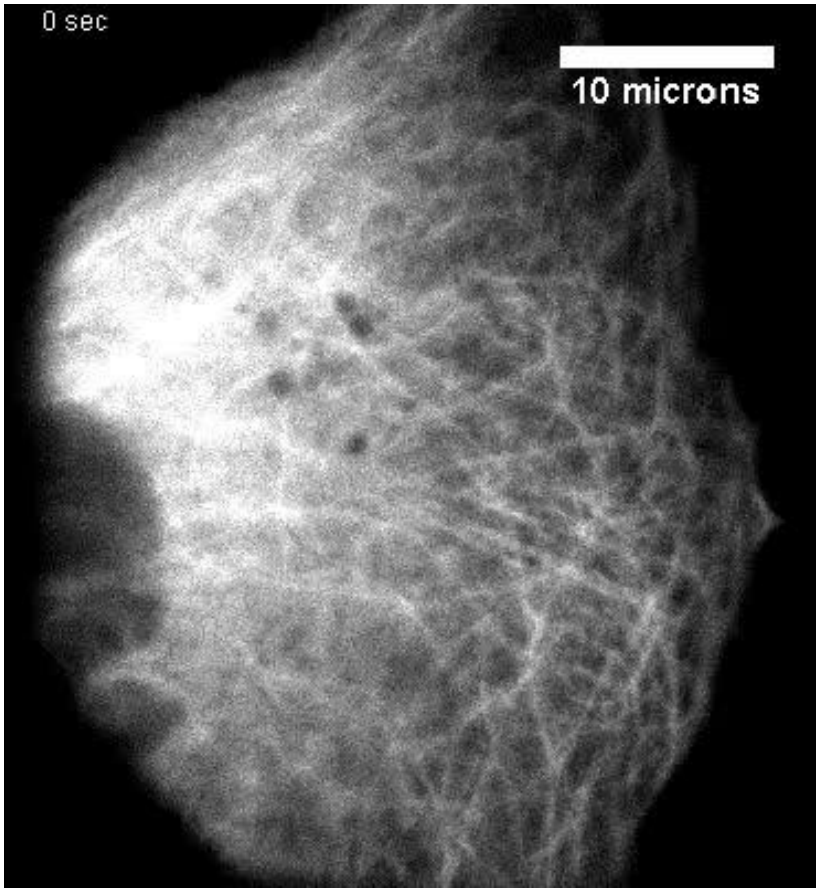
COS 7 cell transfected with GFP-tubulin

Fluctuating motion within cells

Brownian motion



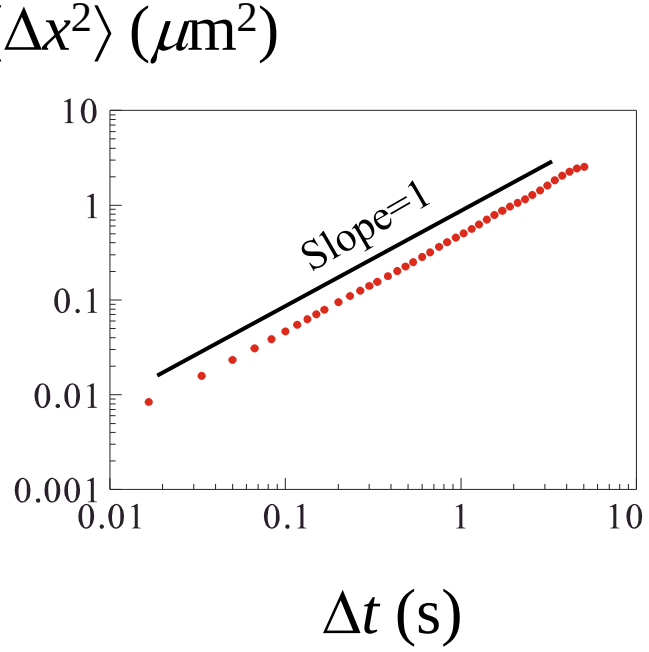
2 μm particles



COS 7 cell transfected with GFP-tubulin

Brownian motion

Mean square displacement:



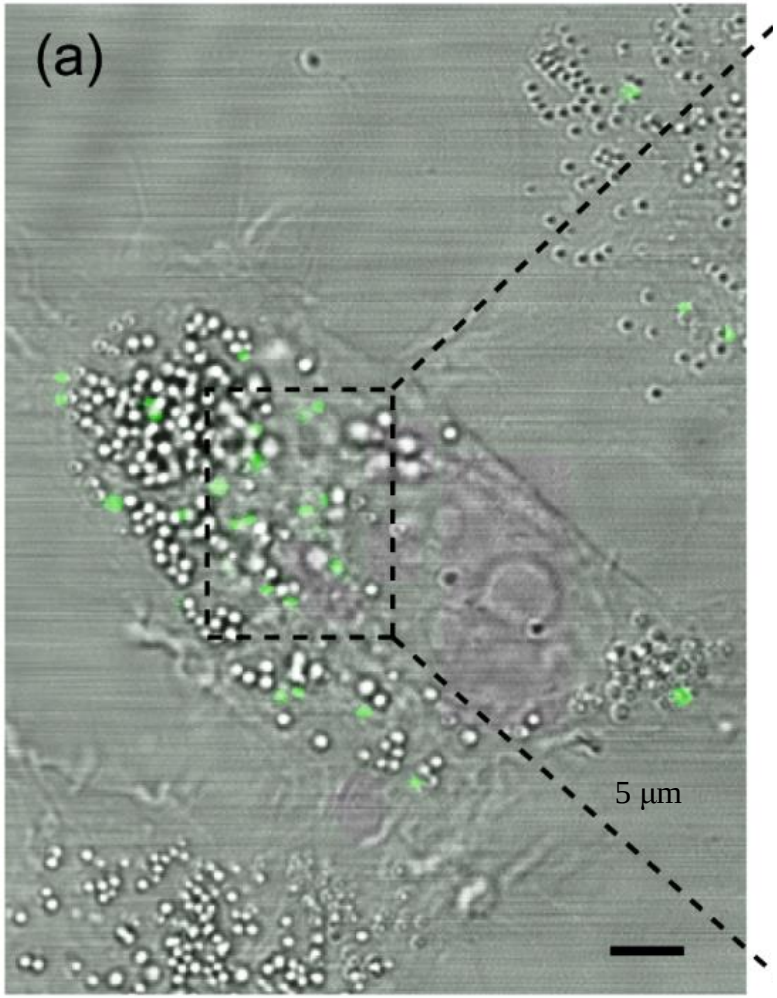
Leads to normal diffusion: $\langle \Delta x^2 \rangle = 2Dt$

$$D = \frac{k_B T}{6\pi\eta a}$$

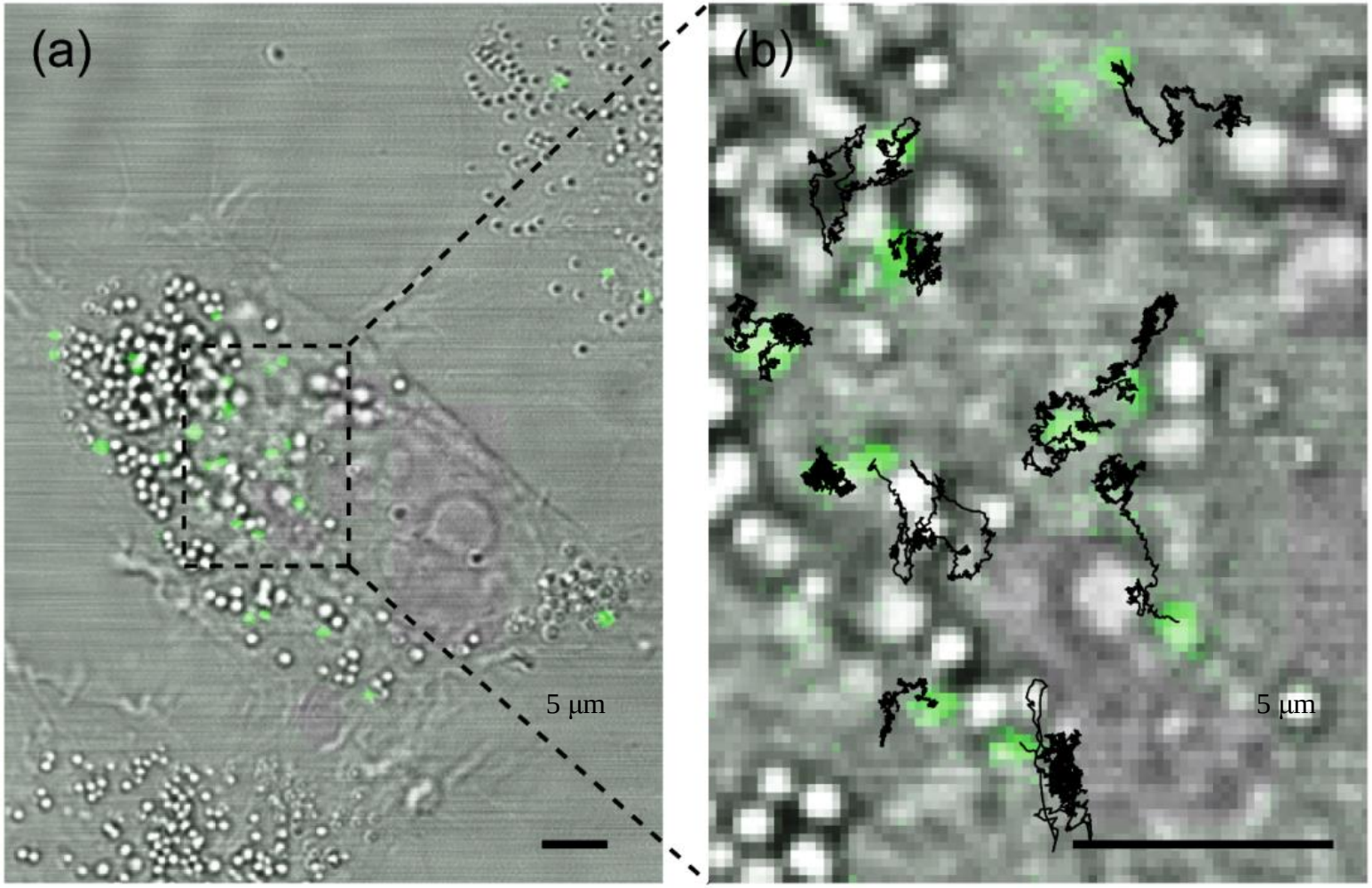
Particle size a

viscosity η

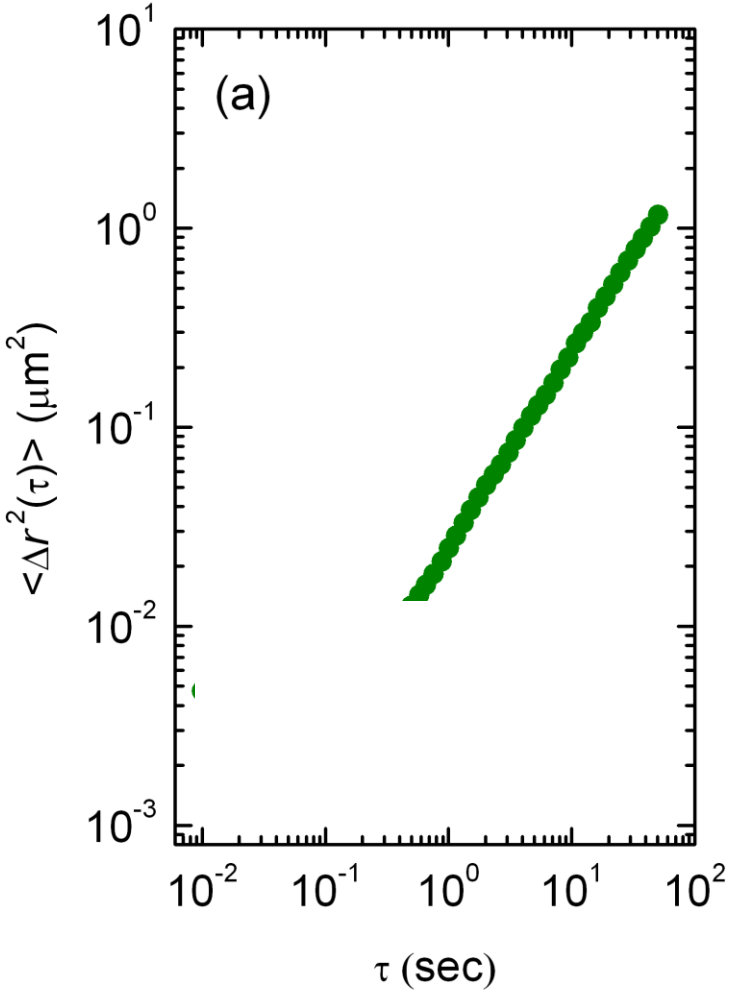
Micro-inject inert beads into cells
Probe motion within a cell



Micro-inject inert beads into cells
Probe motion within a cell



Mean-squared displacement of 200 nm beads in wild type A7 cells

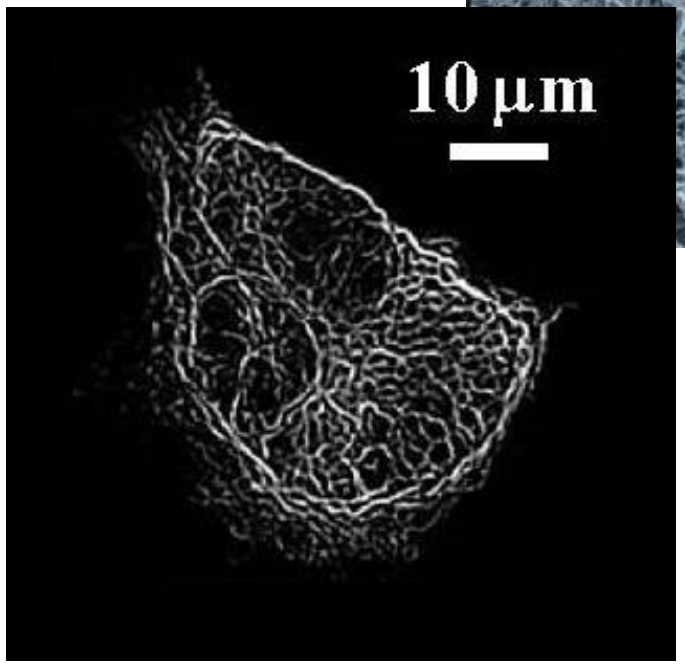


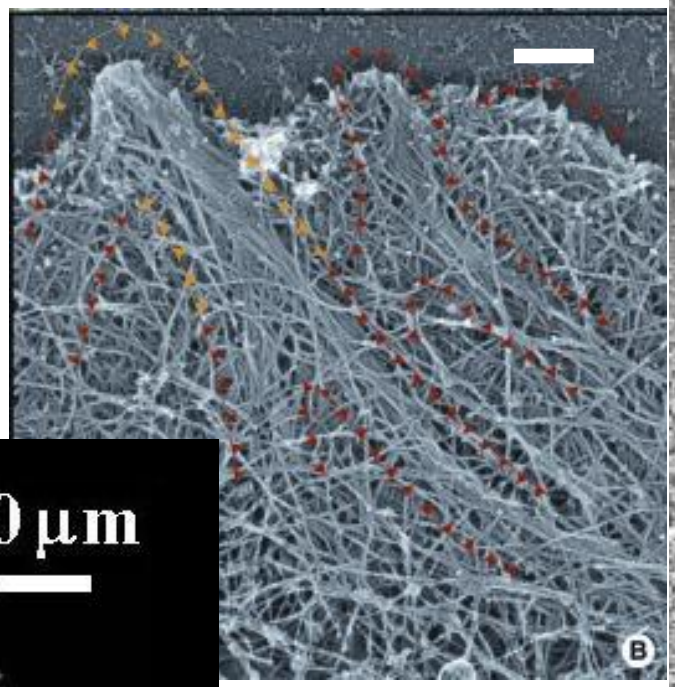
Motion appears diffusive

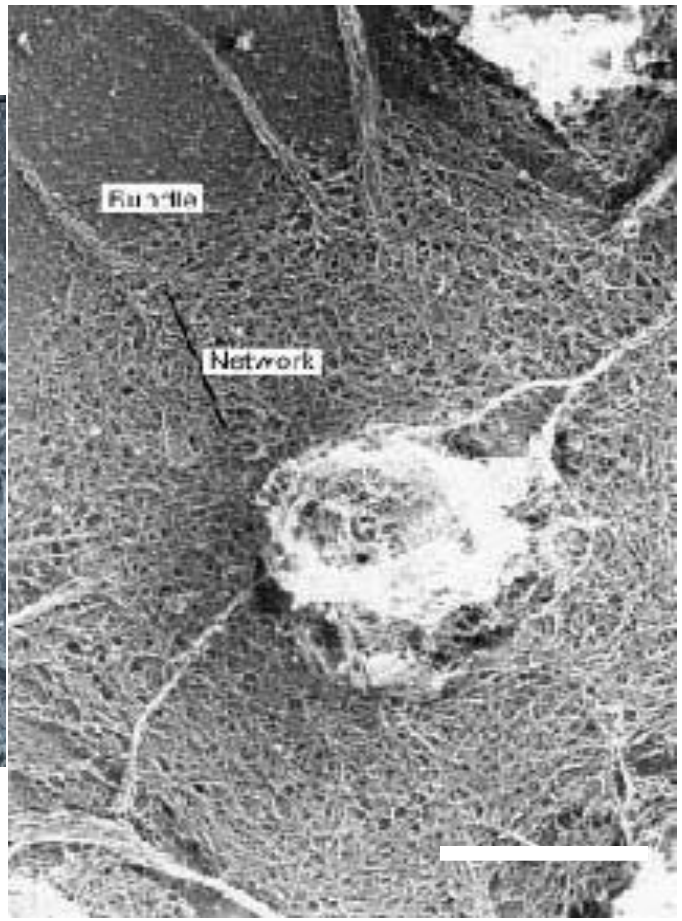
Microrheology:
Viscous medium
η = 500 x water

$$D = \frac{k_B T}{6\pi\eta a}$$

Biopolymer networks in cells

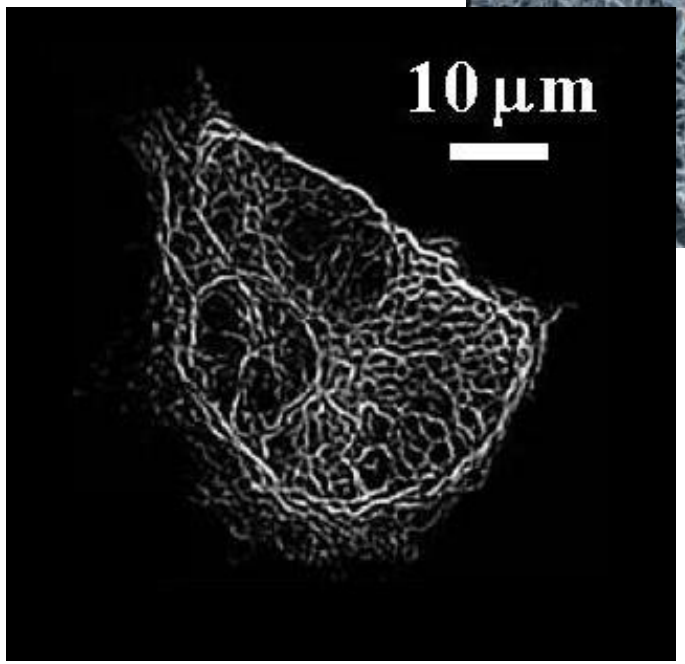




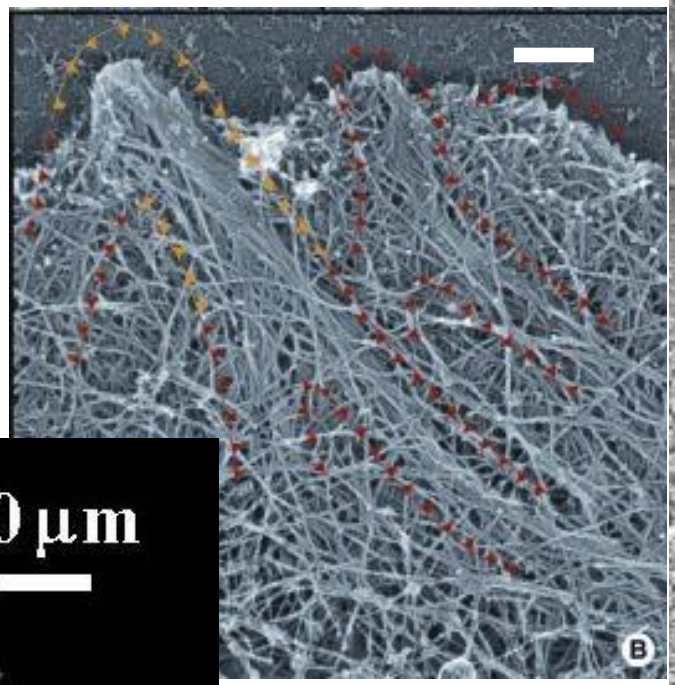


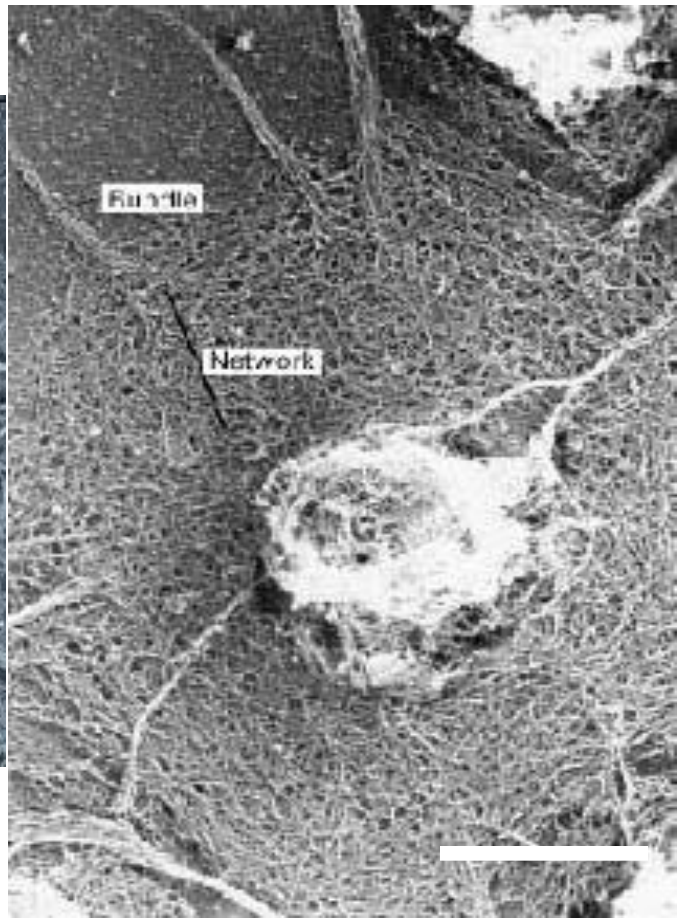
Lead to rigidity in cells

Biopolymer networks in cells



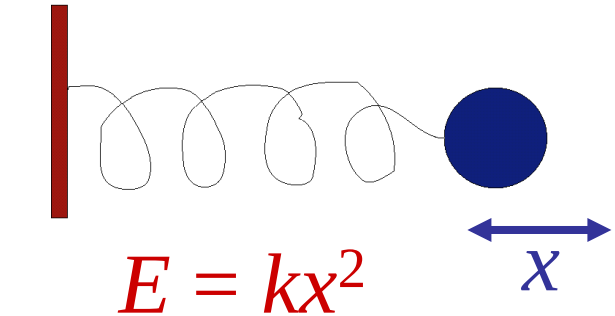
0.2 μm

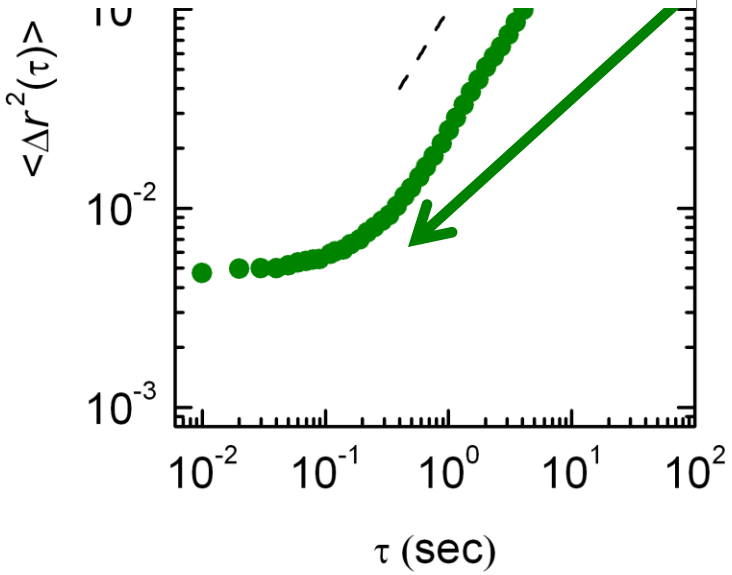




5 μm

MSD of 200nm beads in wild type A7





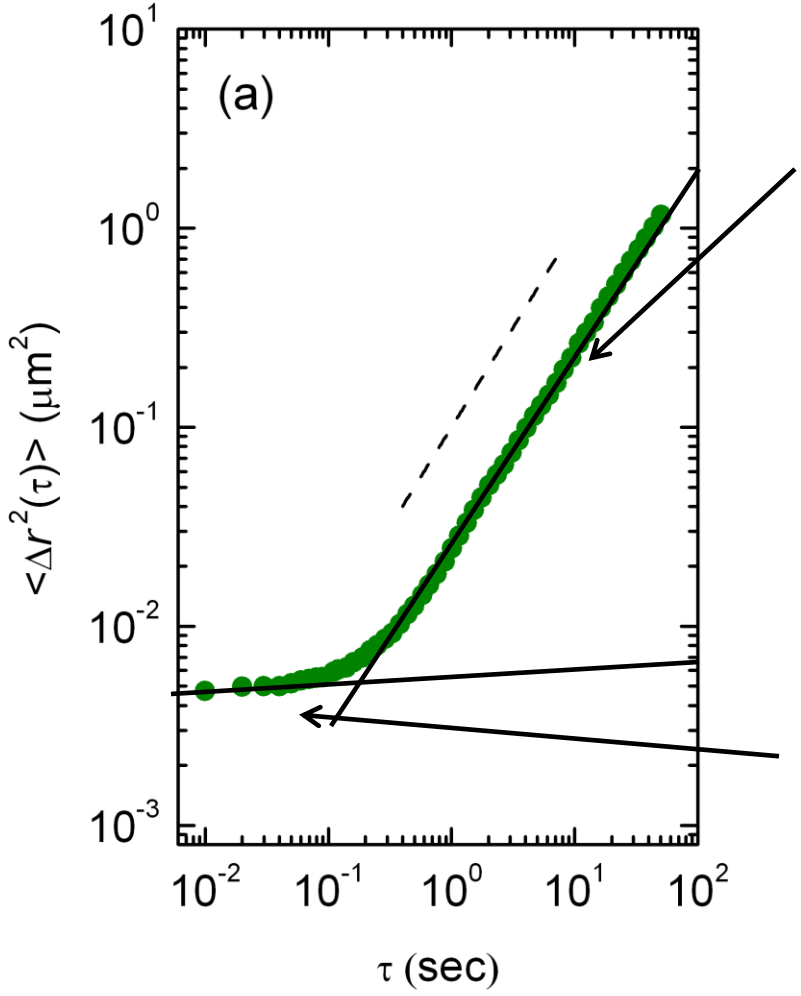
Microrheology:
Elastic medium
Equate thermal energy
to
Motion of particle on spring

$$k_B T \sim \frac{1}{2} k \left\langle \Delta r^2(t) \right\rangle$$

G = 1Pa

$$G_0' = \frac{2k_B T}{3\pi a \left\langle \Delta r^2(\tau) \right\rangle}$$

MSD of 200nm beads in wild type A7



$$\left\langle \Delta r^2(\tau) \right\rangle = \frac{2k_B T}{3\pi a \eta} t$$

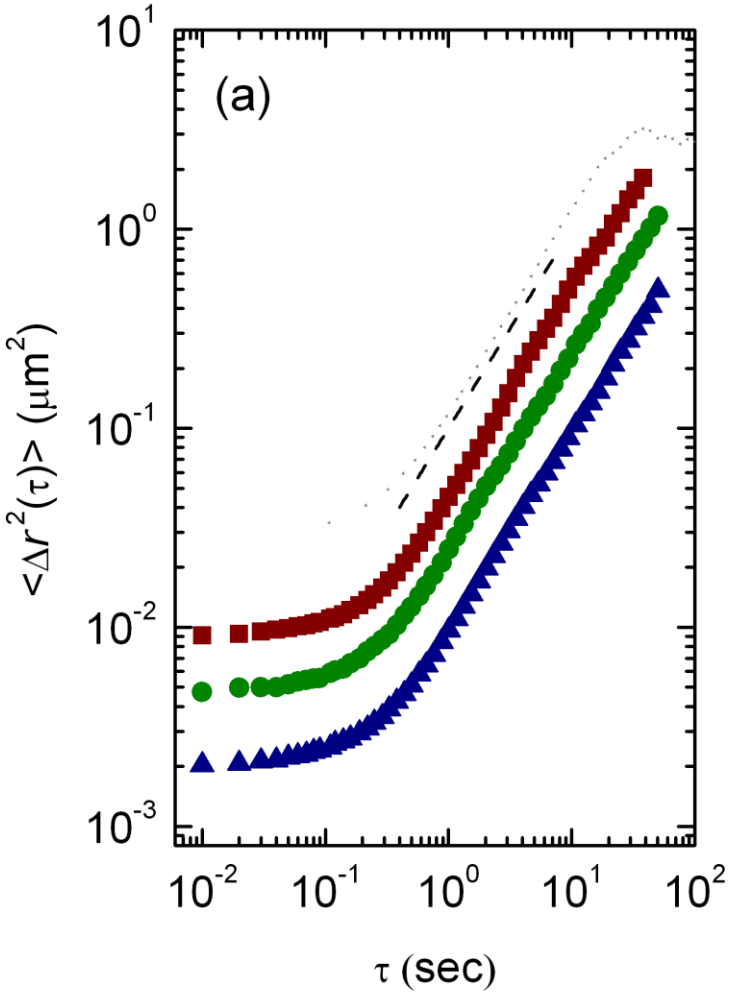
Viscous medium

Microrheology

$$\left\langle \Delta r^2(\tau) \right\rangle = \frac{2k_B T \tau}{3\pi a \eta} = \frac{2k_B T}{3\pi a G_0'}$$

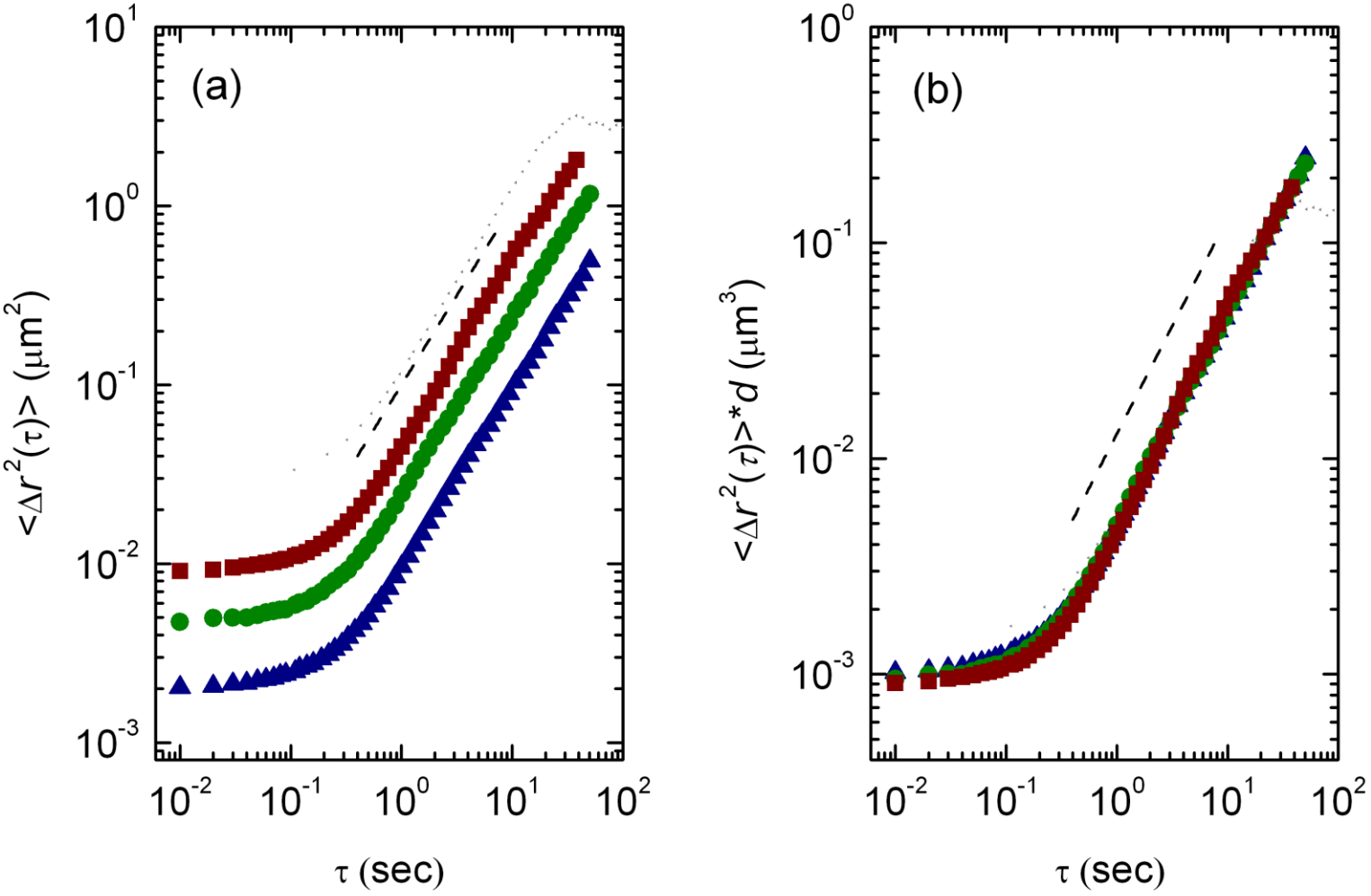
Elastic medium

MSD of beads in wild type A7



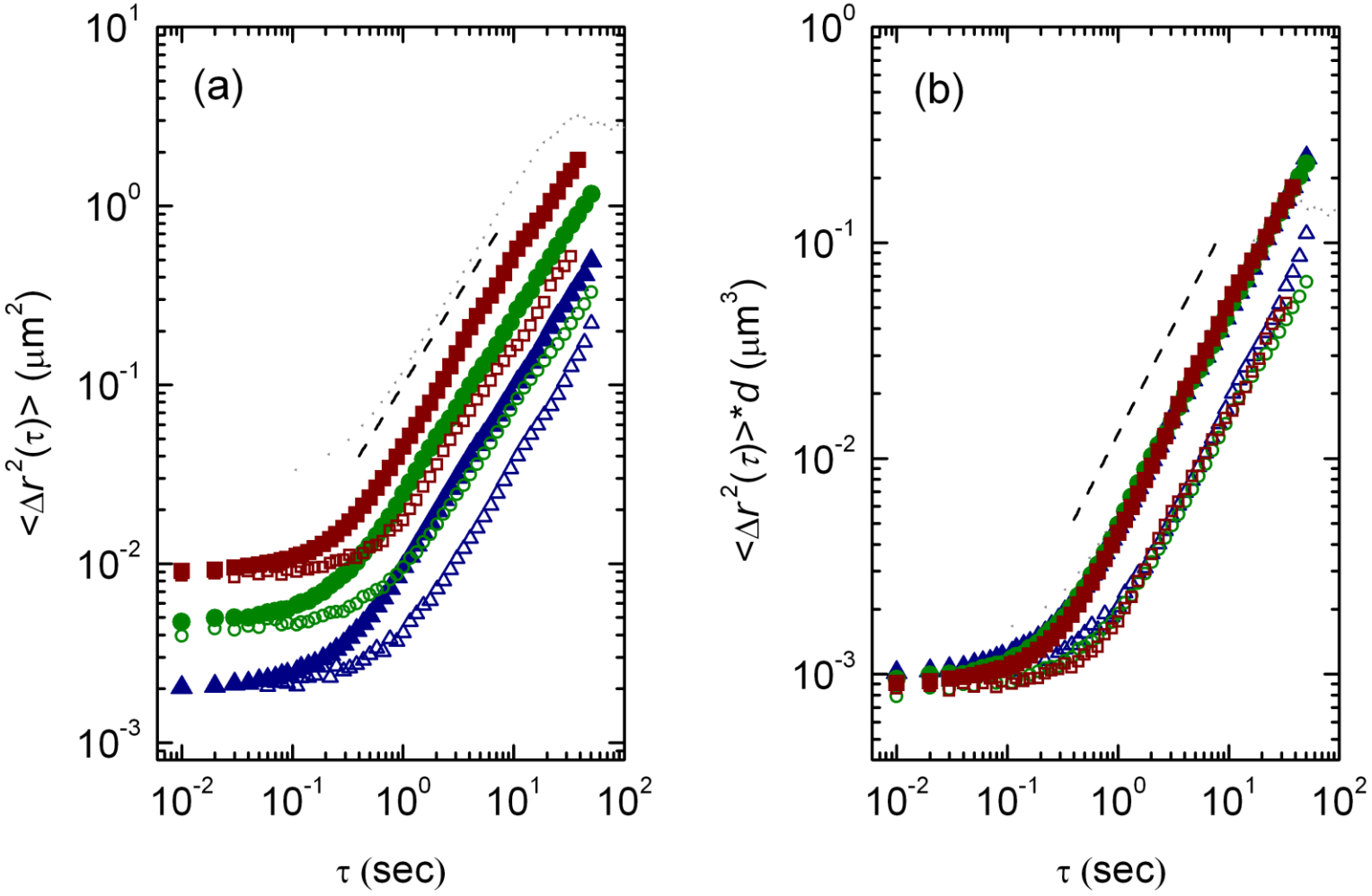
Red: 100nm in diameter
Green: 200nm
Blue: 500nm

Scale by bead radius



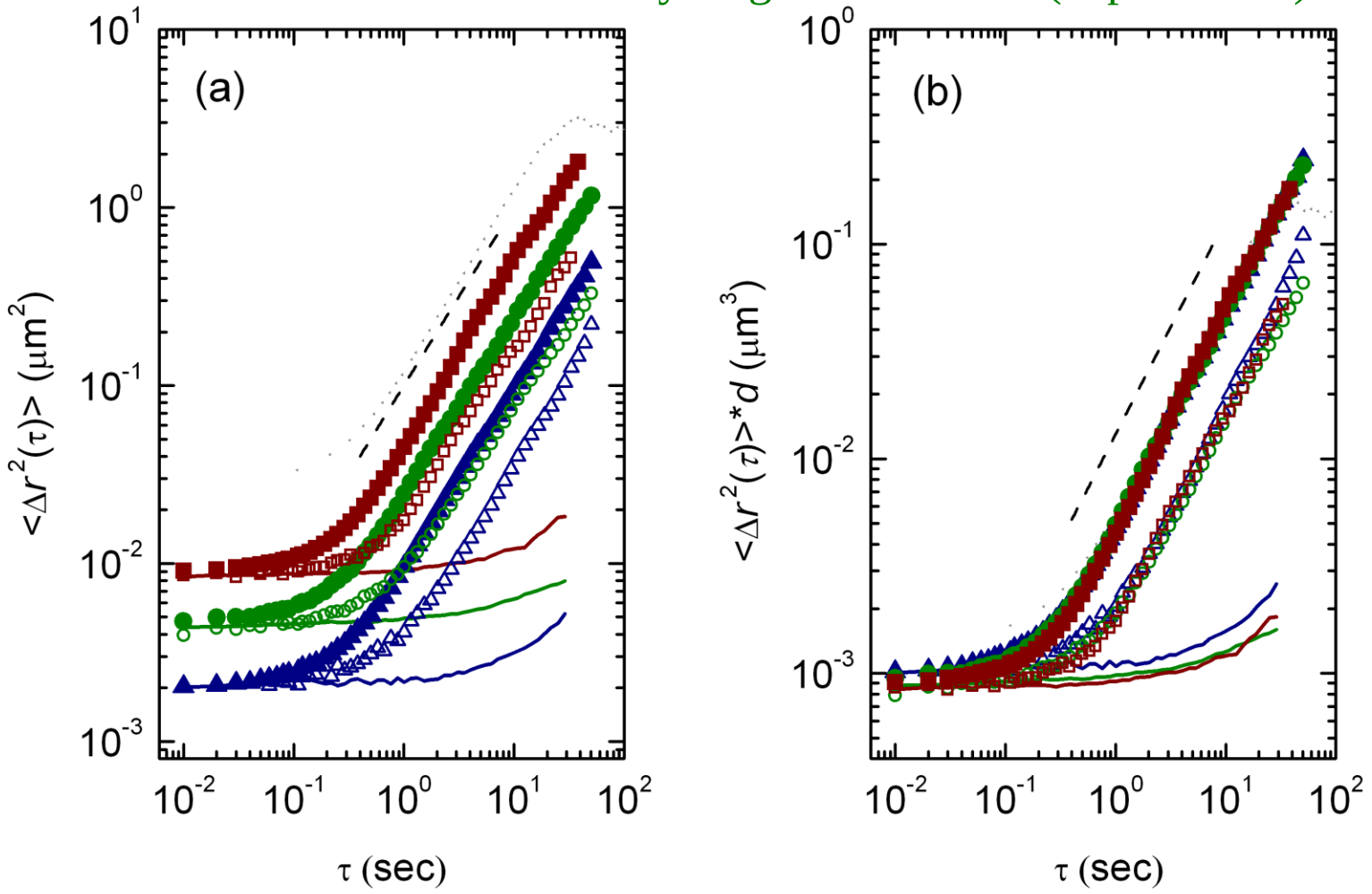
Decrease cell's activity

Open symbols: 10 μM Blebbistatin treated (inhibit Myosin II motors)

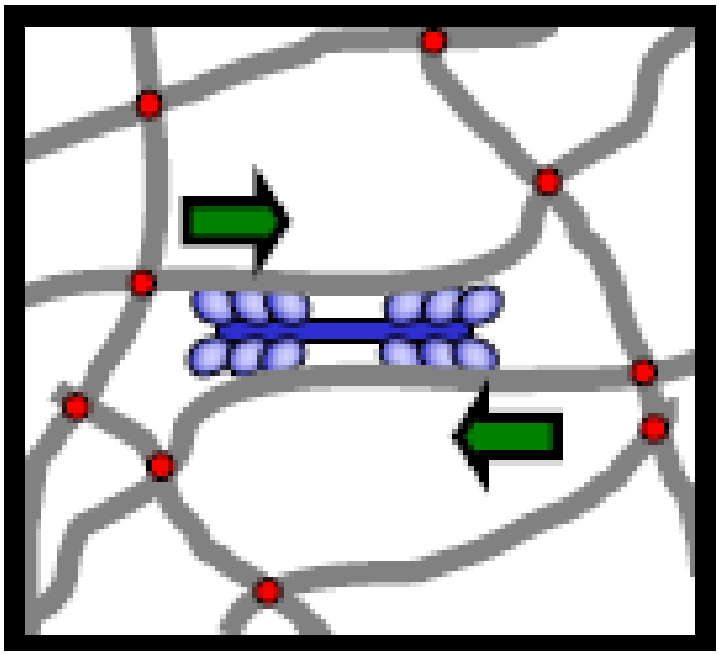


Further decrease in cell activity

Open symbols: 10 μM Blebbistatin treated (inhibit Myosin II motors)
Lines: Sodium Azide + 2-deoxy-D-glucose treated (deplete ATP).

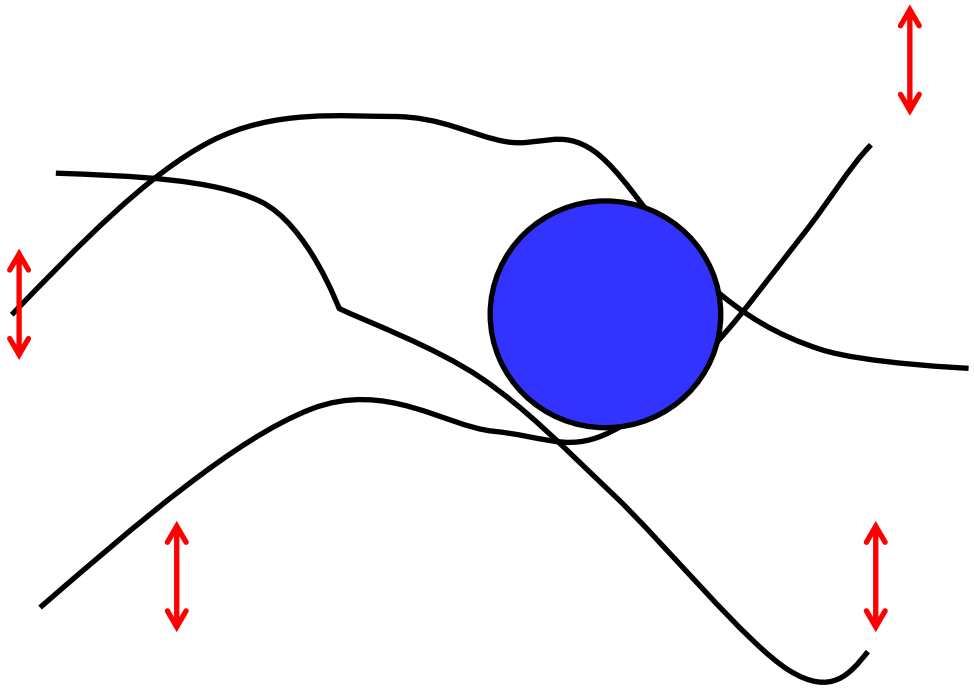


Effect of molecular motors on microtubules



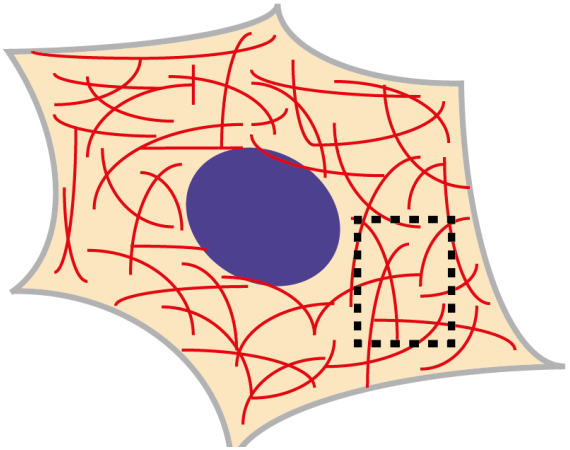
Requires a solid network

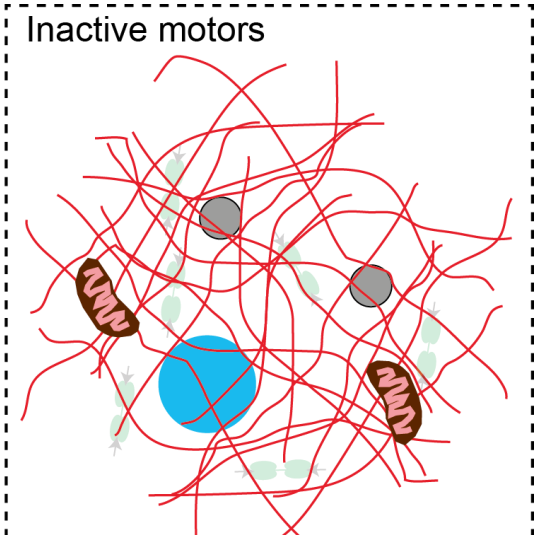
Fluctuations of elastic network



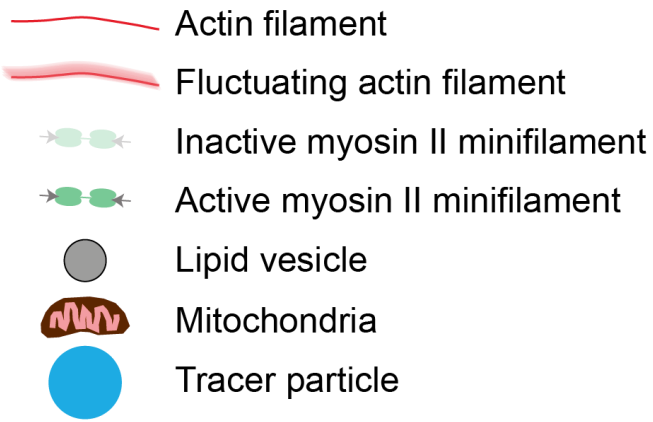
- Levine & MacKintosh; Lau *et al*
- Elastic network
- Random forces from motors
- Random force dipoles drive fluctuations

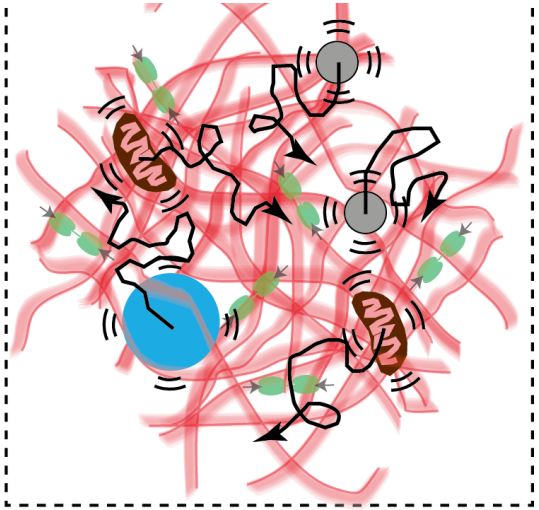
Distribution of motors → random forces



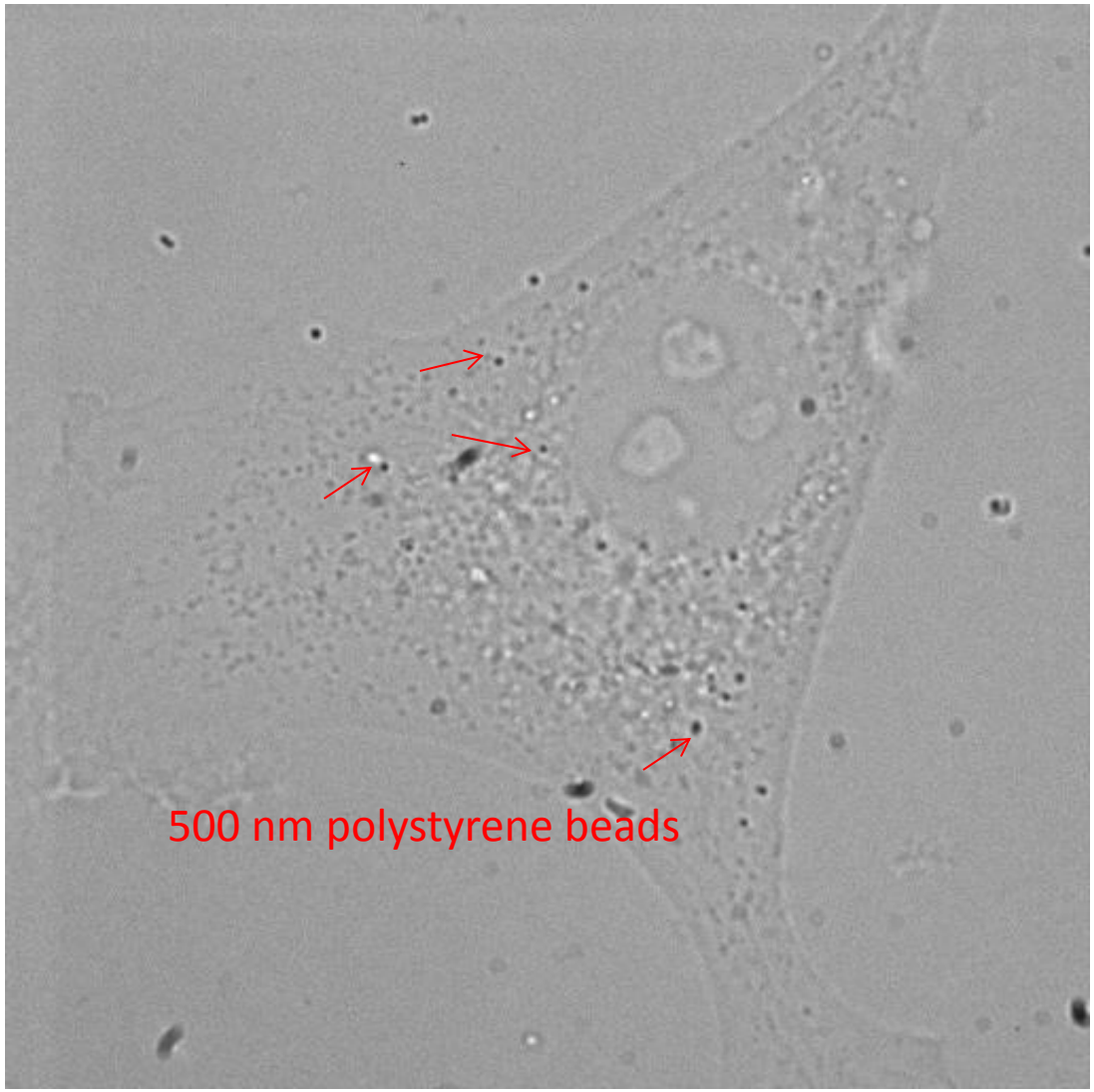


Particle must be in an elastic matrix





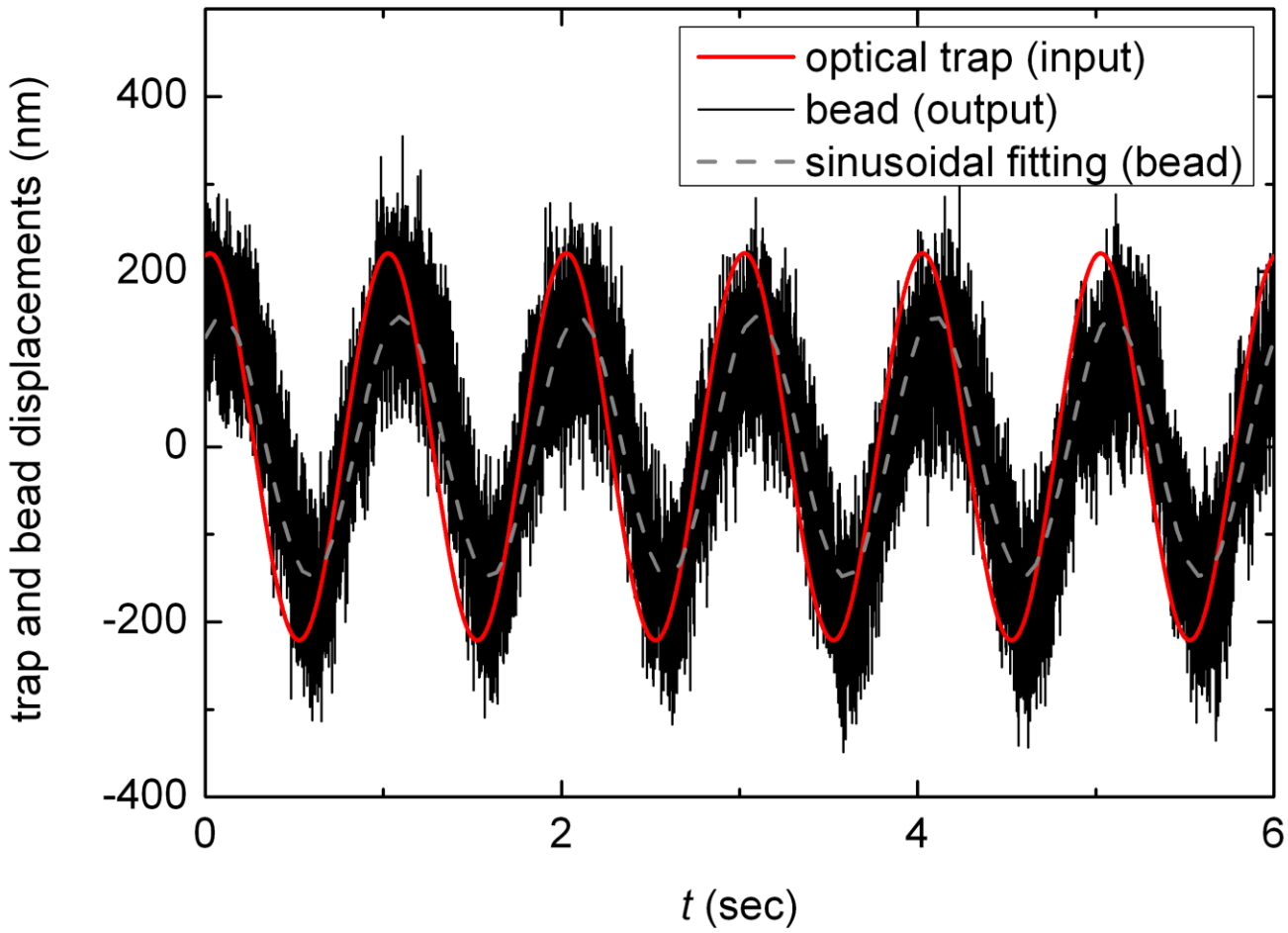
Active microrheology



Jeff Moore
Mikkel Jensen
BU

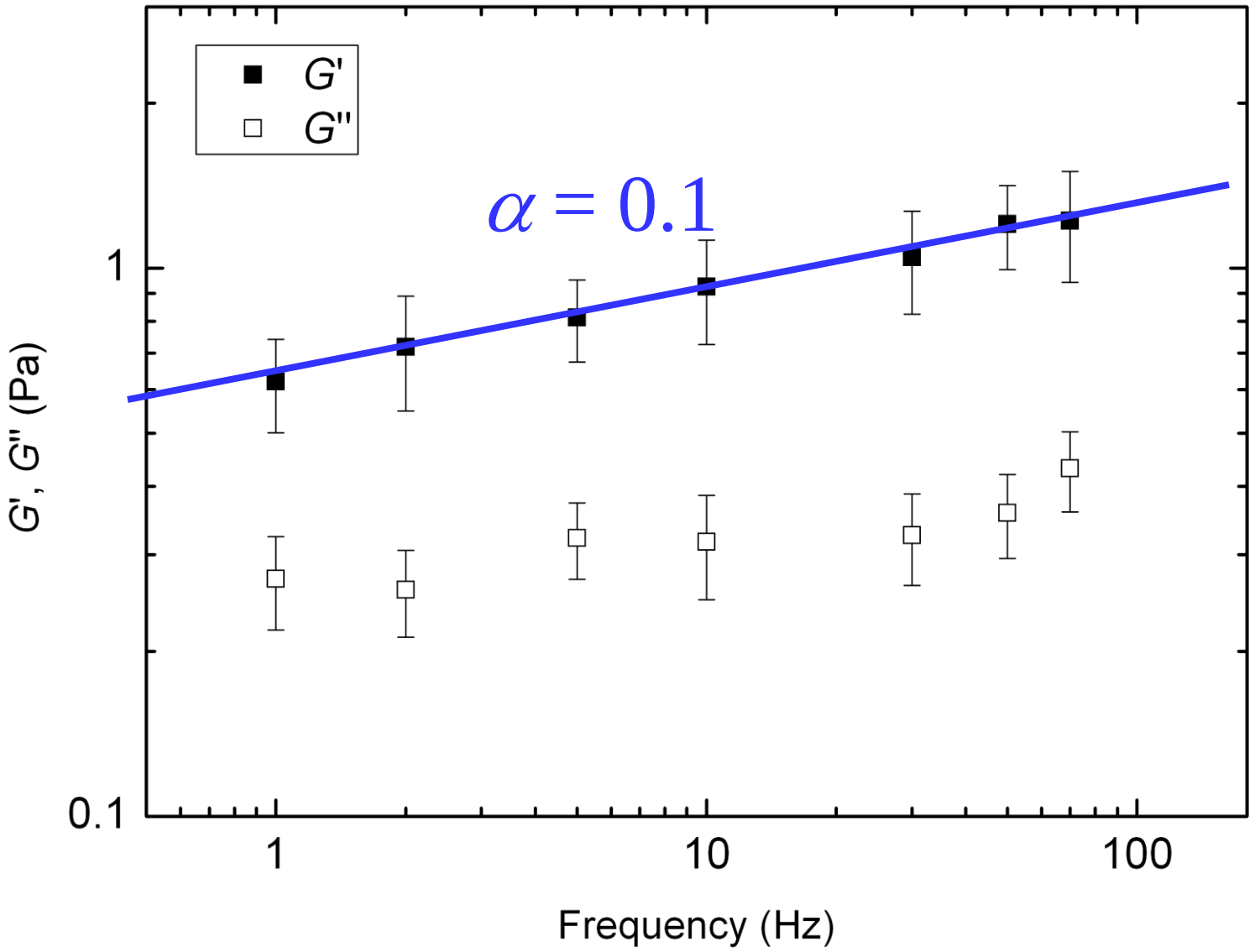
Laser tweezers

Optical Trap Oscillation and Bead Response (f = 1 Hz)

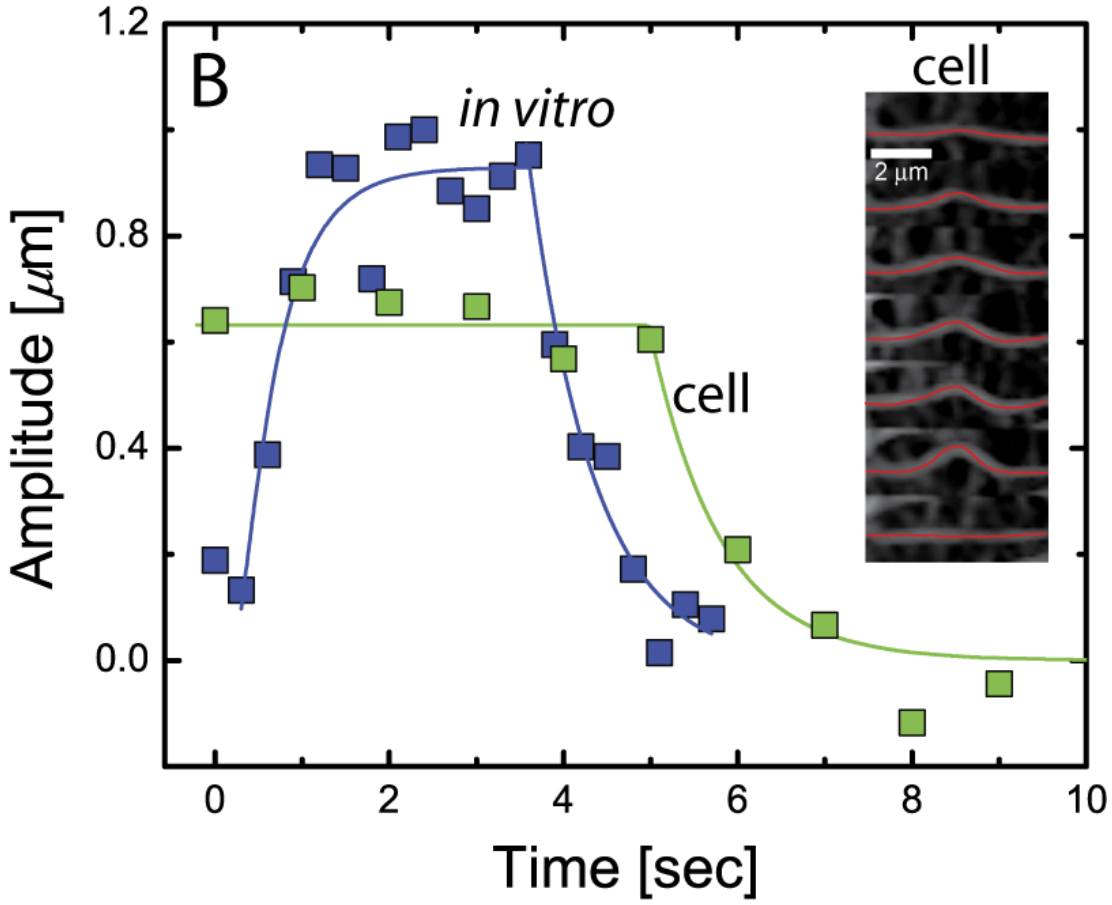


Response is still very much in phase at f=1Hz, suggests that the material inside is still elastic at 1Hz.

Active microrheology



Spectrum of forces on microtubules



Exponential time dependence

Fluctuations of elastic network

$$\left\langle x^2(\omega)\right\rangle = \frac{\left\langle f^2(\omega)\right\rangle}{\left|K(\omega)\right|^2}$$

Force spectrum due to active motors

$$\left\langle f_{\text{act}}^2(\omega)\right\rangle \propto \frac{1}{\omega^2 + 1/\tau_{\text{p}}^2}$$

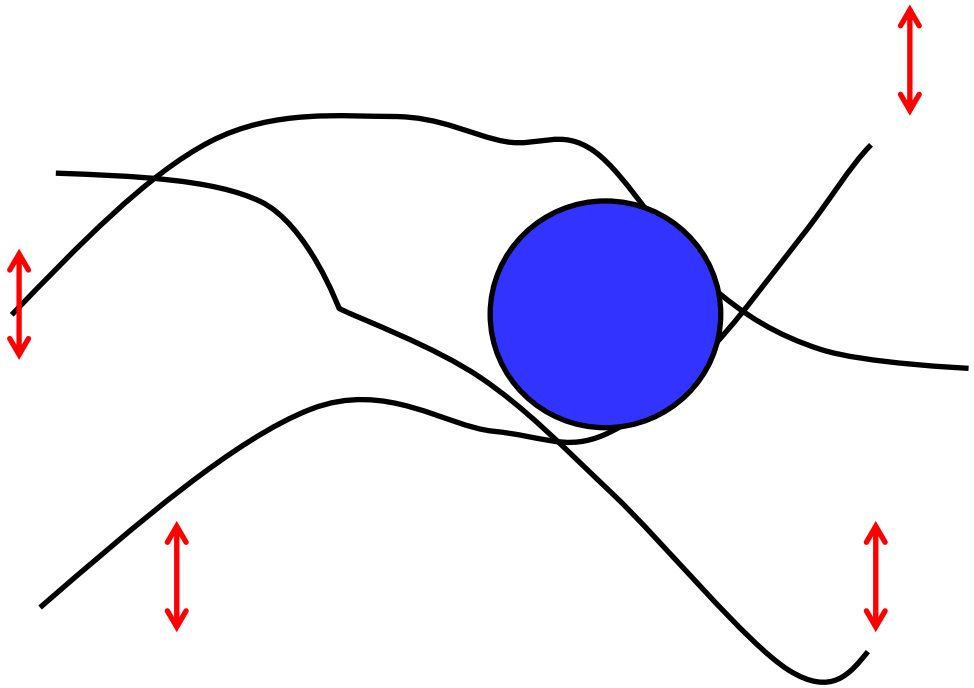
Viscoelastic response

$$\left\langle \Delta r^2 \right\rangle \sim t$$

$$\left\langle \Delta r^2 \right\rangle \sim t^{2\alpha}$$

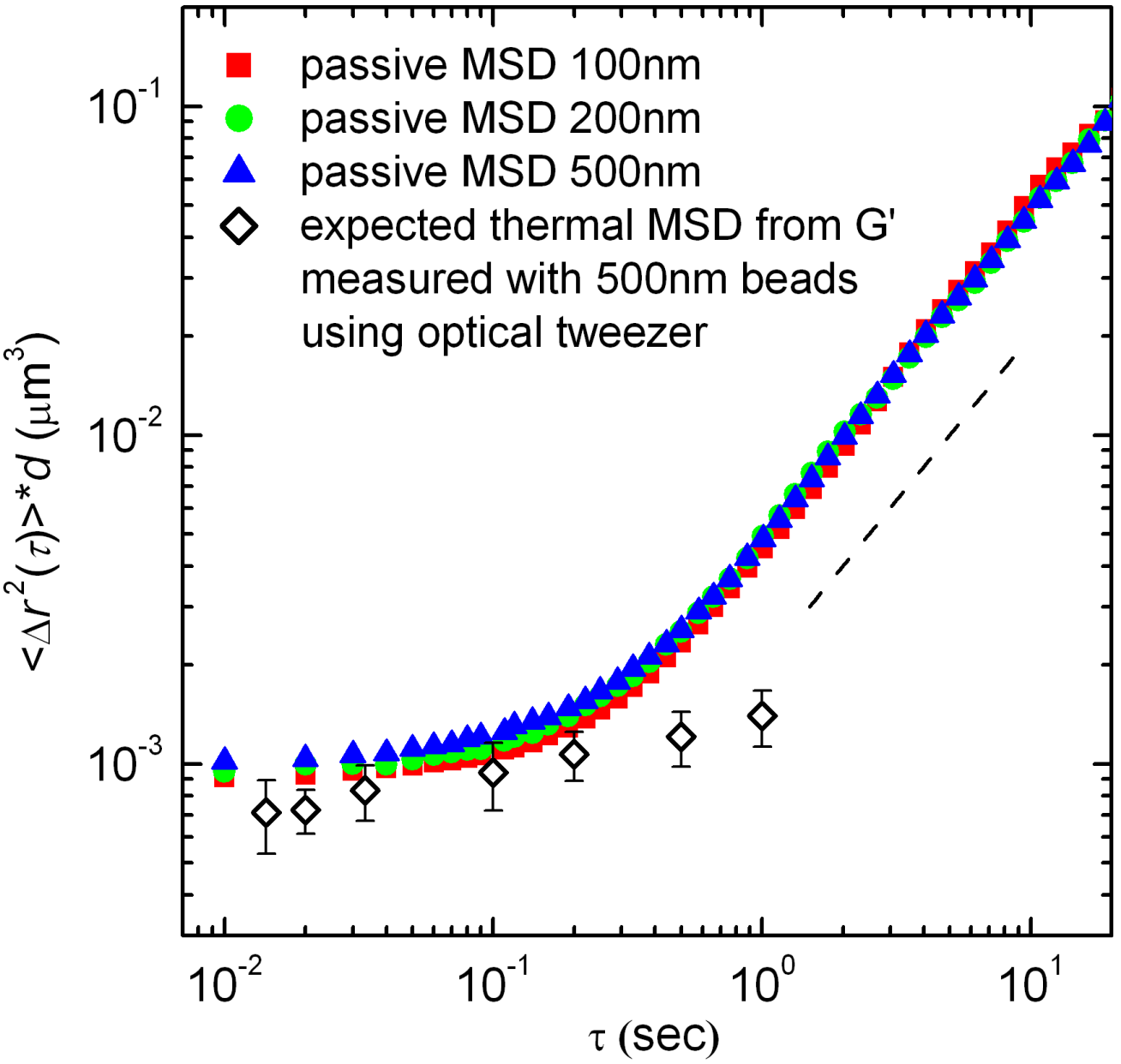
$$\left\langle \Delta r^2 \right\rangle \sim t^{1.2}$$

Fluctuations of elastic network

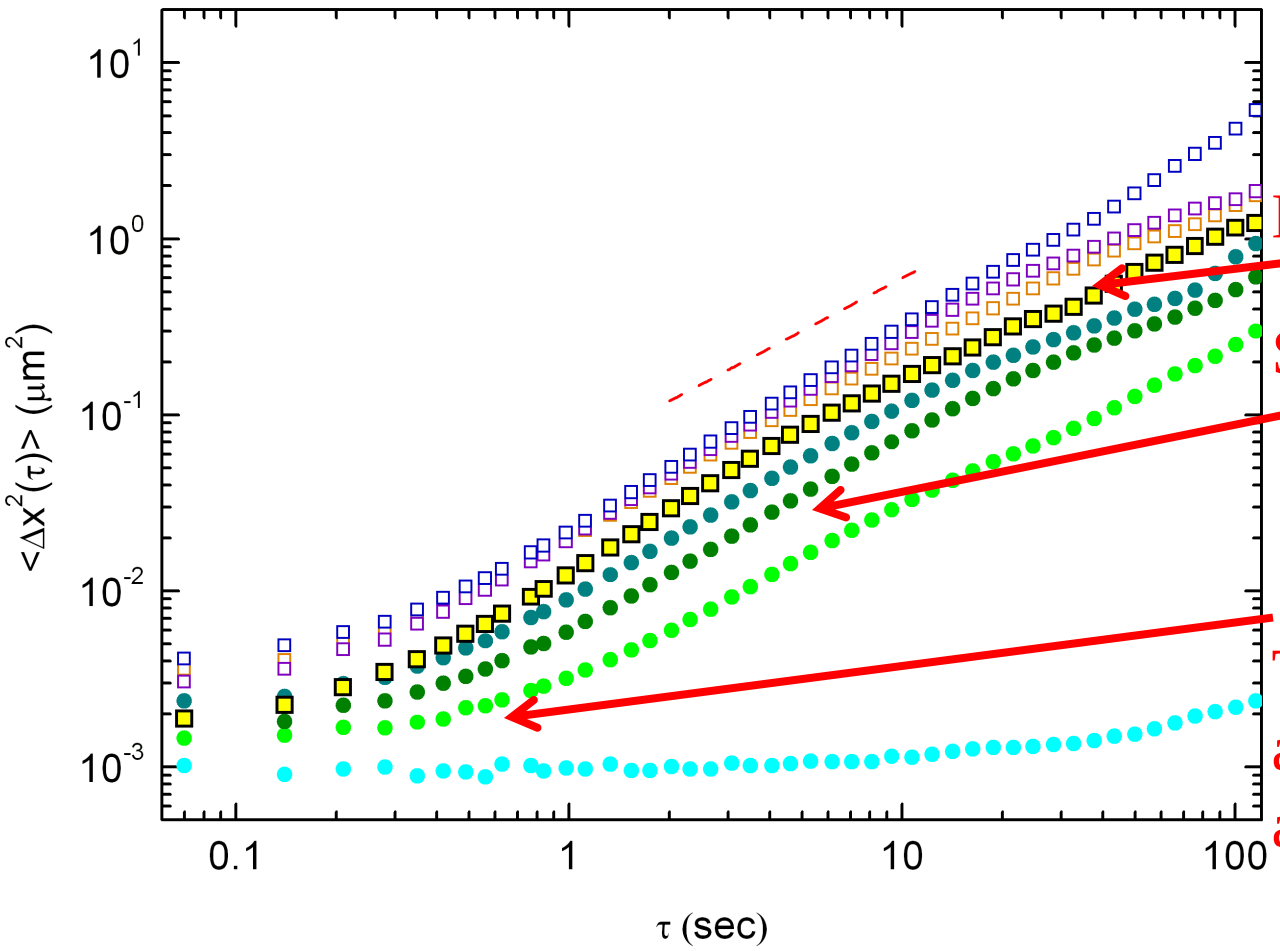


- Elastic network
- Random forces from motors
- Random force dipoles drive fluctuations

Scaled Mean Square Displacement



Fluctuations of elastic network



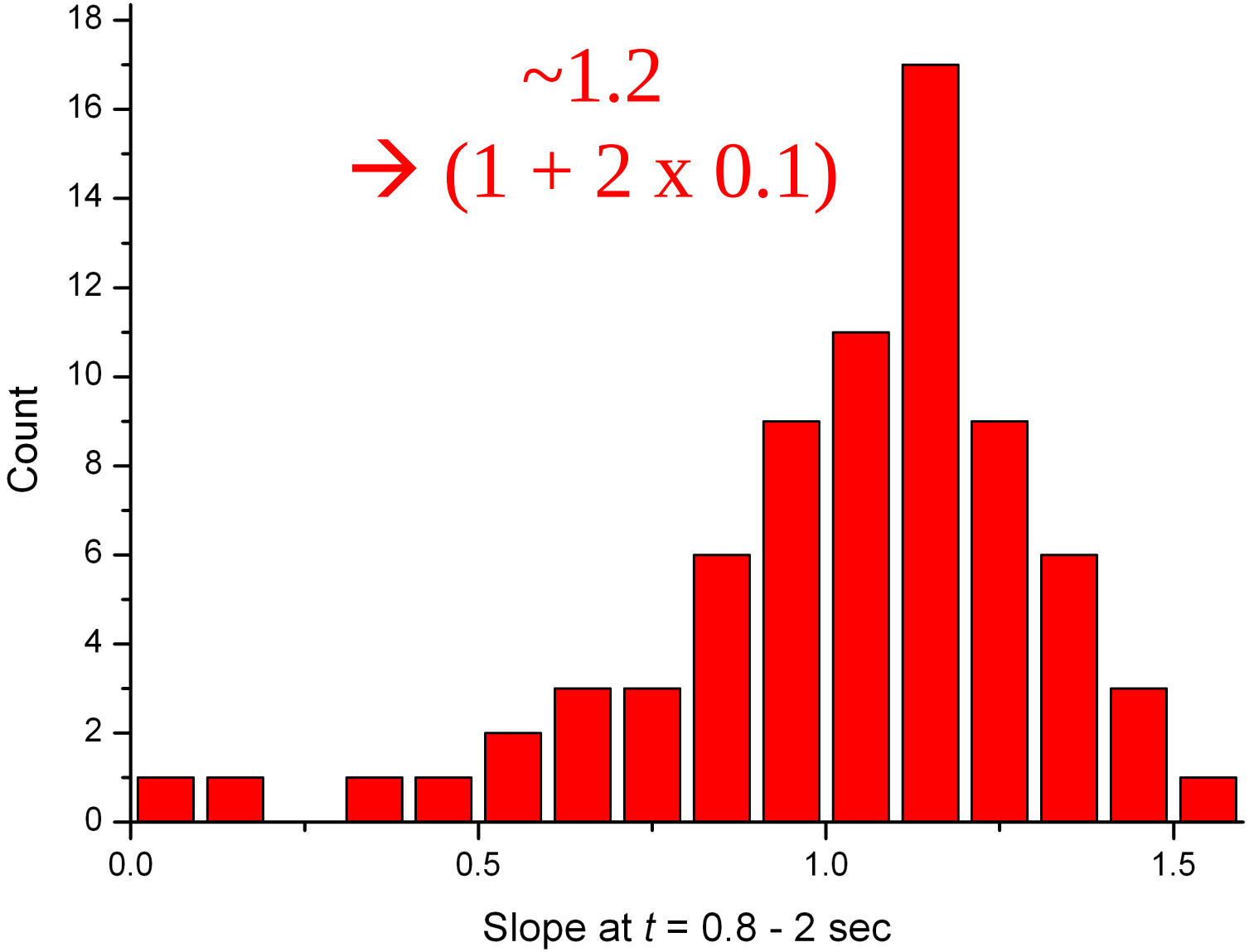
Motor processivity time

Slopes greater than 1

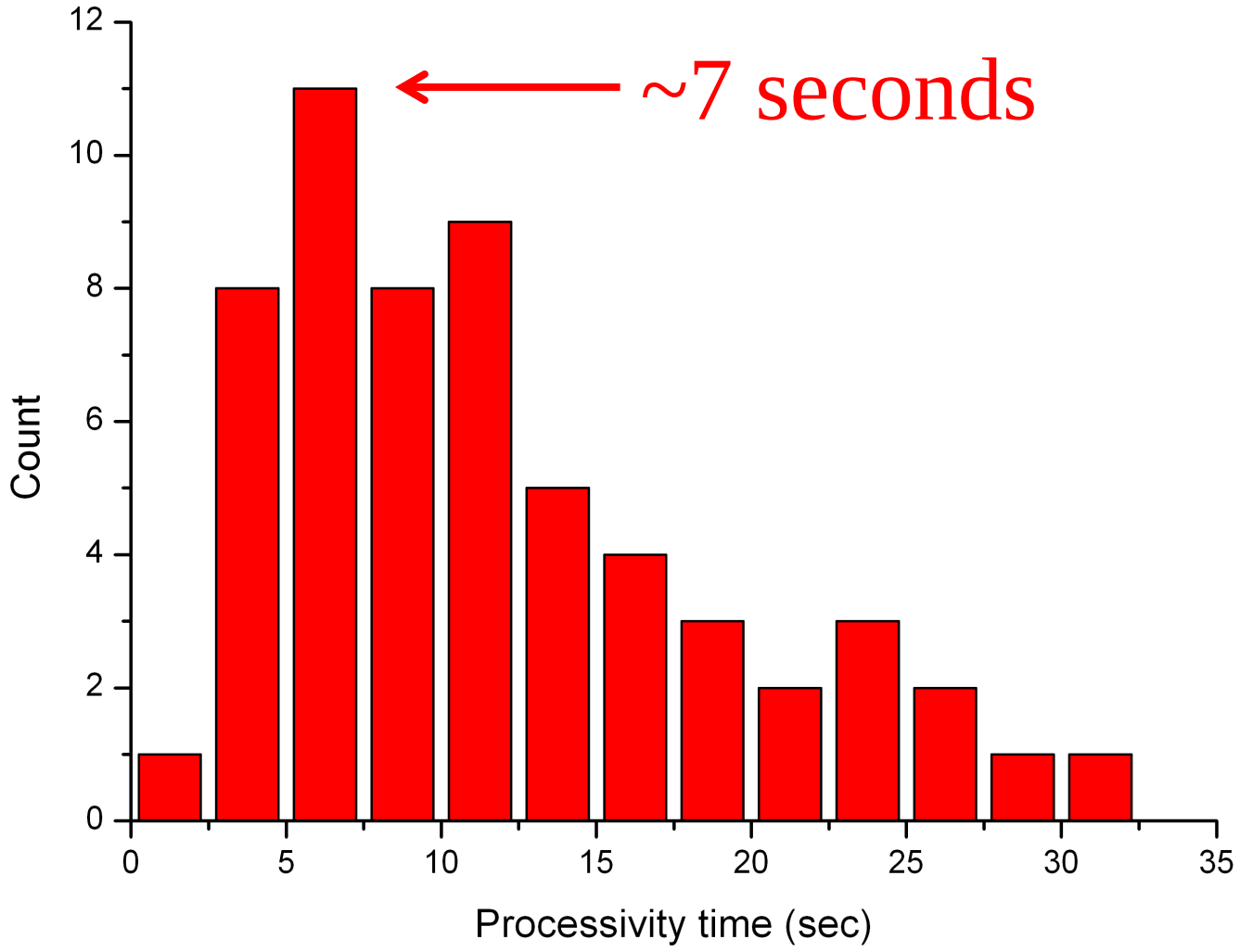
Time when Brownian and motor fluctuations are equal

- Slope: 1+2α:
  - 1 from motor fluctuations
  - α from elastic behavior of network

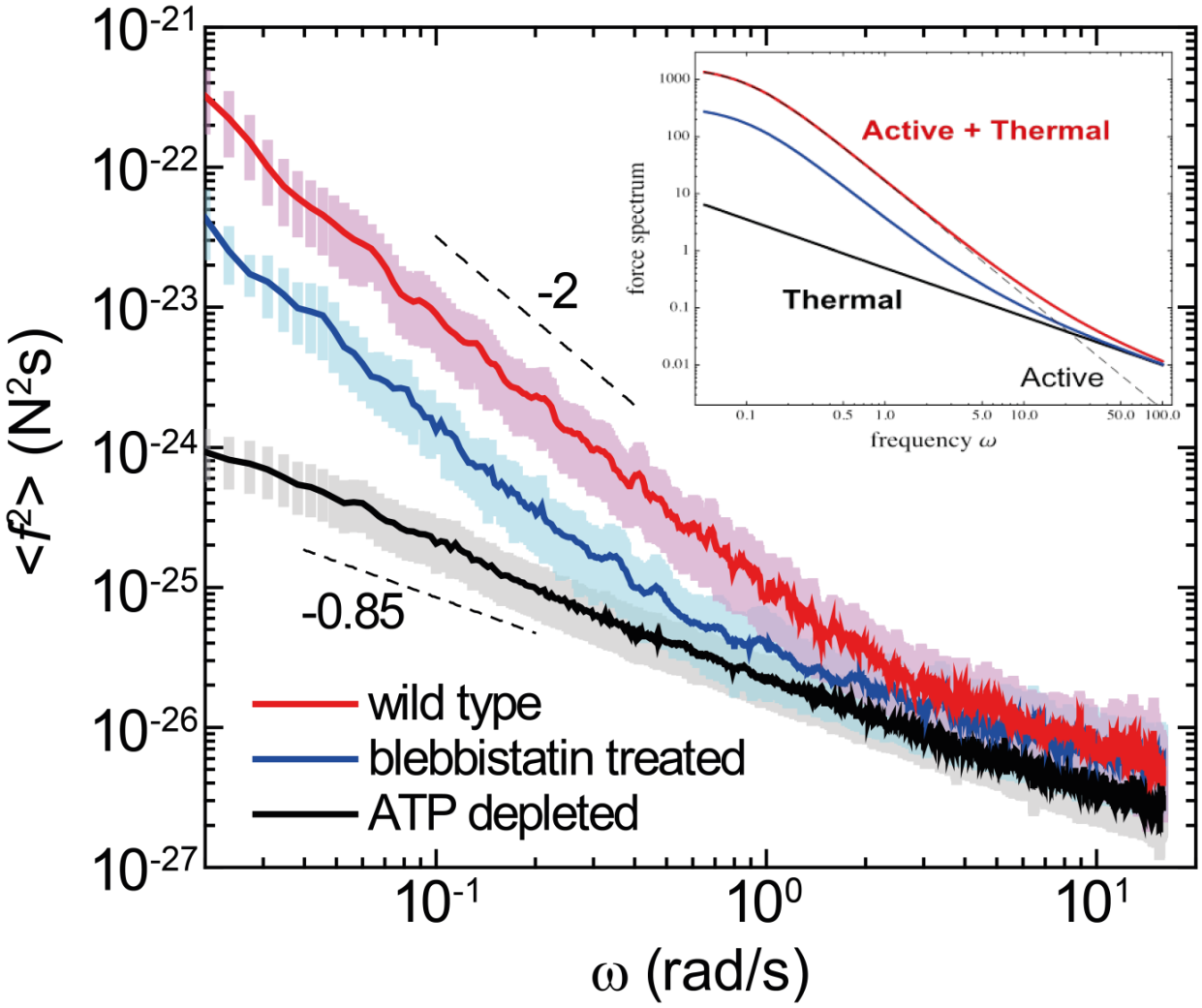
Distribution of local slopes



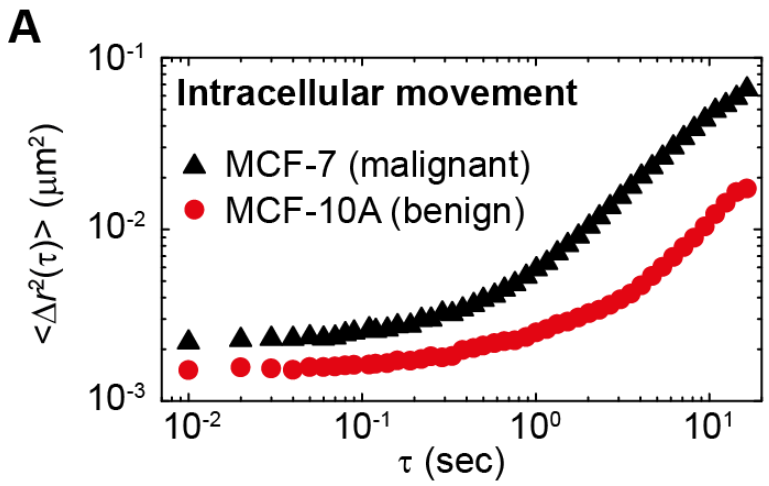
Distribution of processivity times



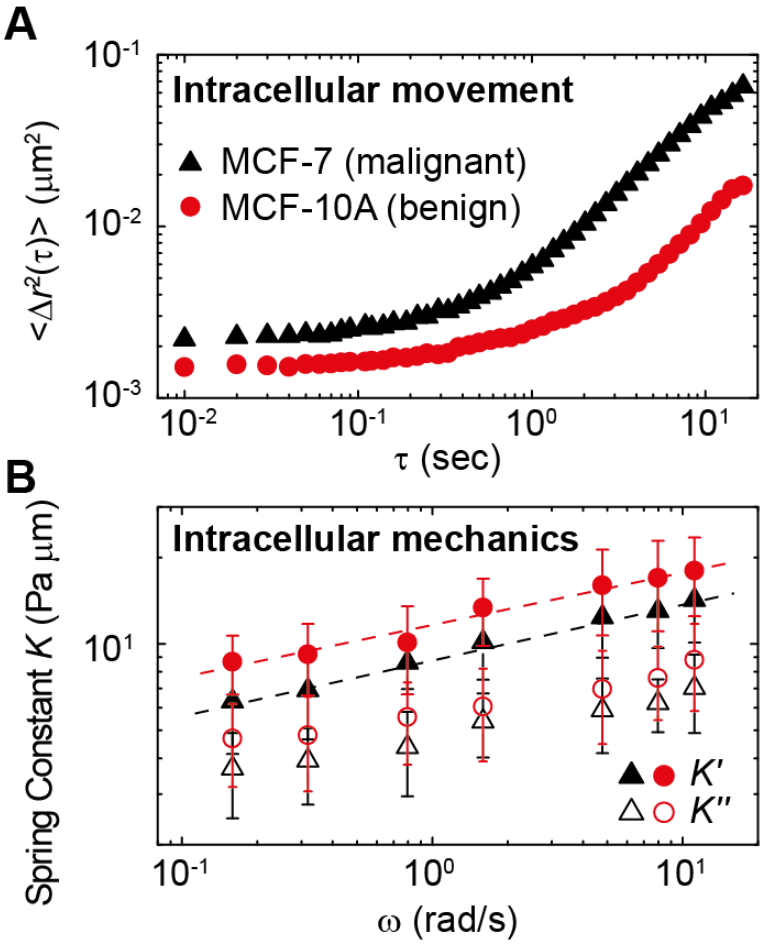
Force spectrum: Colored noise



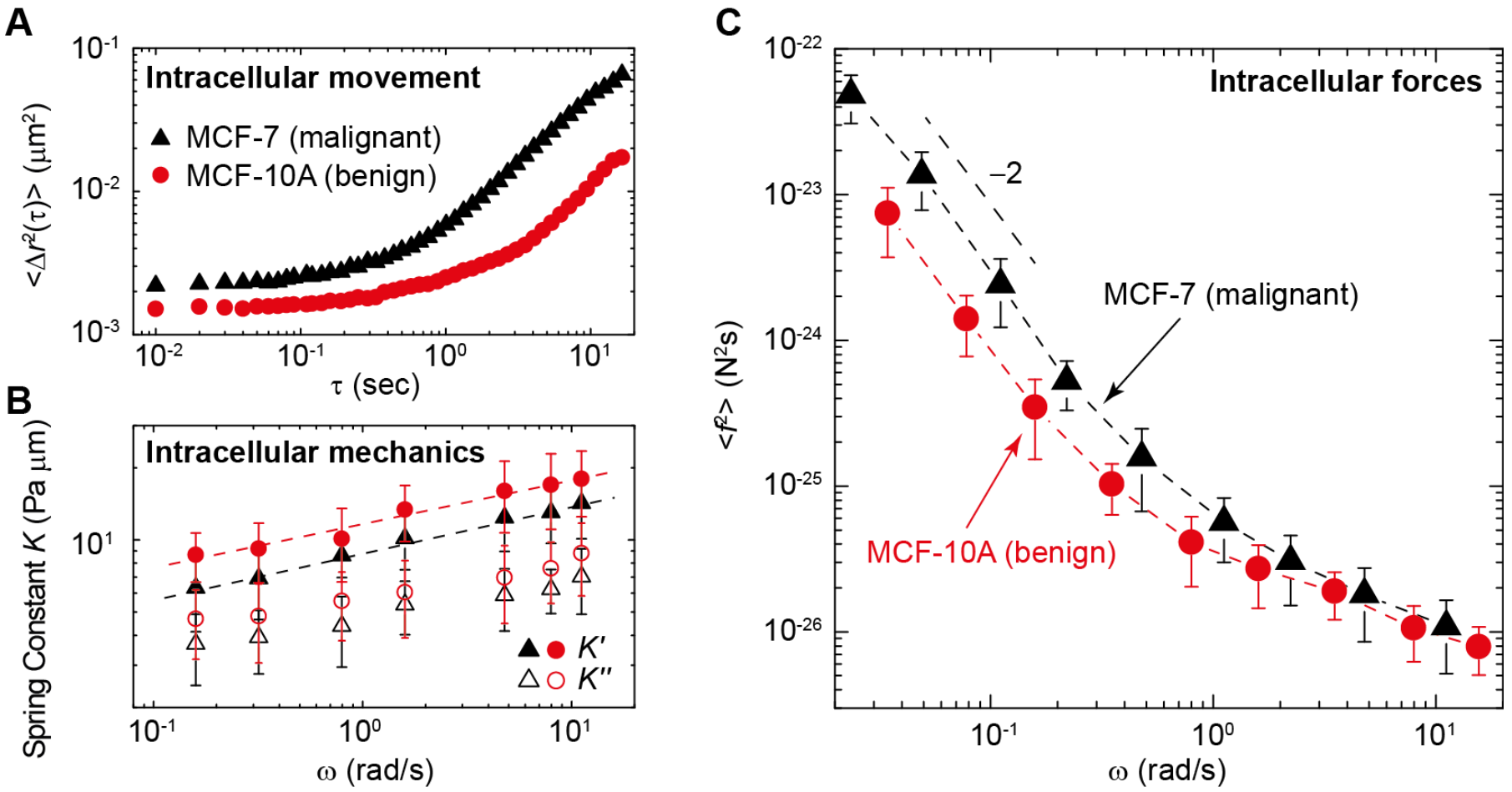
Force spectrum microscopy



Force spectrum microscopy



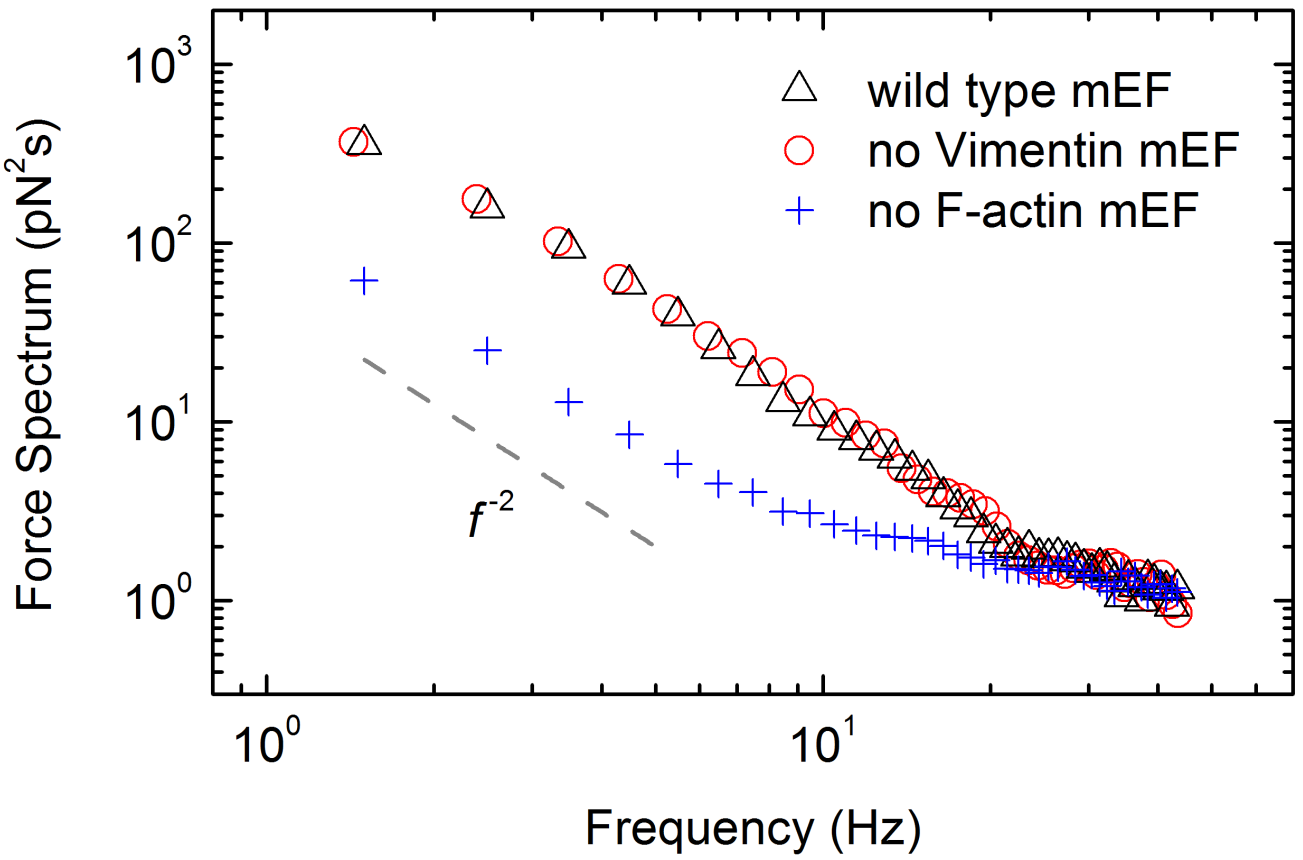
Force spectrum microscopy



New assay to probe cancer cells

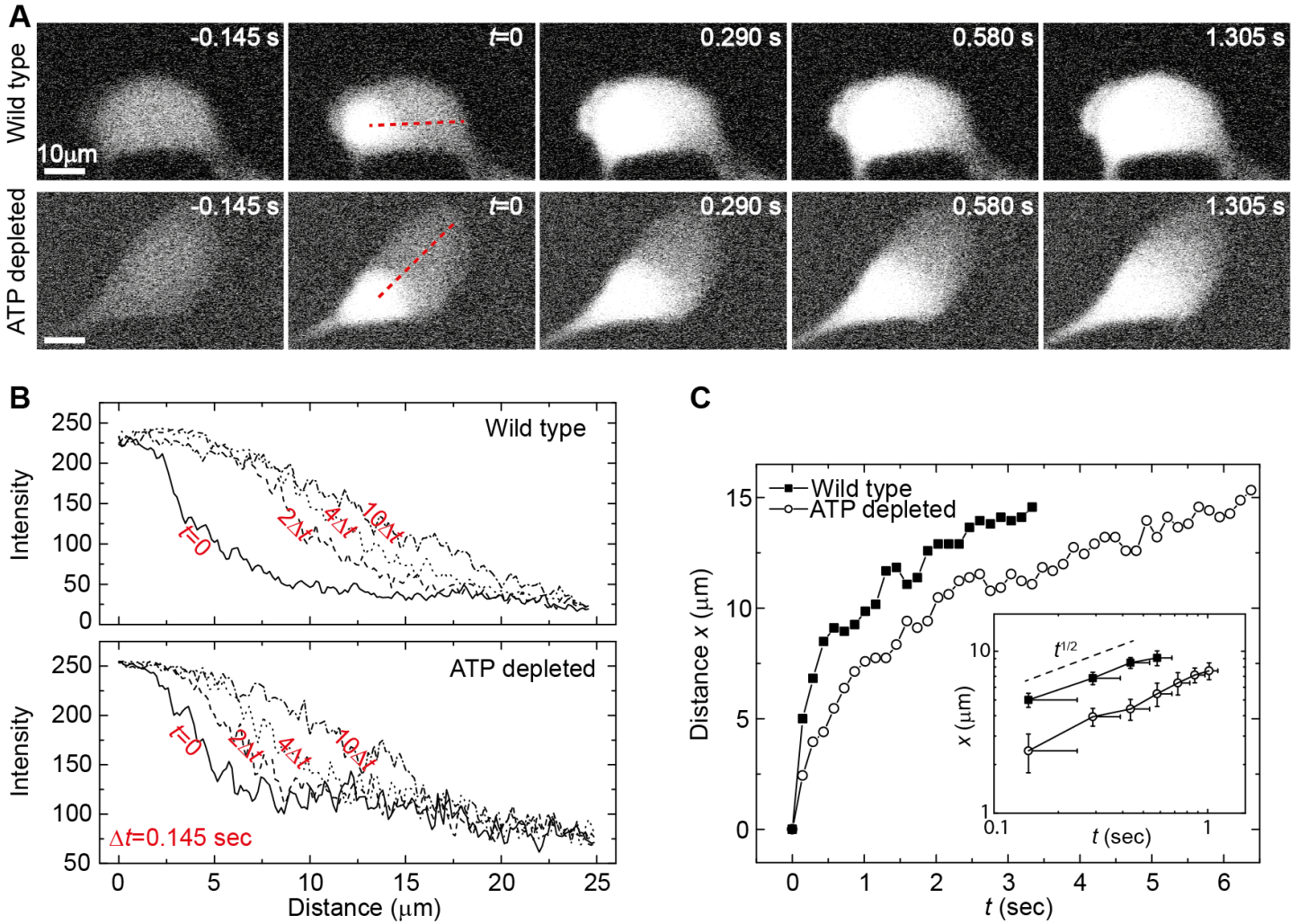
Force spectrum

Test effects of changes of biopolymer networks



cytoD to depolymerize actin

Effect on small molecule transport



Transport of small molecules is enhanced by motor activity.

Effect of motors

- Motors throughout cell
  - Coupled through network, fluid
- Active material
- Leads to ‘effective’ thermal-like behavior
  - Not like thermal behavior in elastic medium
- Elastic medium → depends on *a*
- Lau, Hoffman, Davies, Crocker, Lubensky PRL ’03
- Levine & MacKintosh, PRL ‘09
- Motors impact stiffness of cells

Conclusions

- Biopolymer networks are elastic
- Biopolymer networks are highly non-linear
- Motors produce required internal tension
- Networks and motors cause transverse fluctuations of microtubules
- Motors drive random fluctuations of particles in cells

Thank you for your attention