Measurements and Models of Cytoskeletal Rheology

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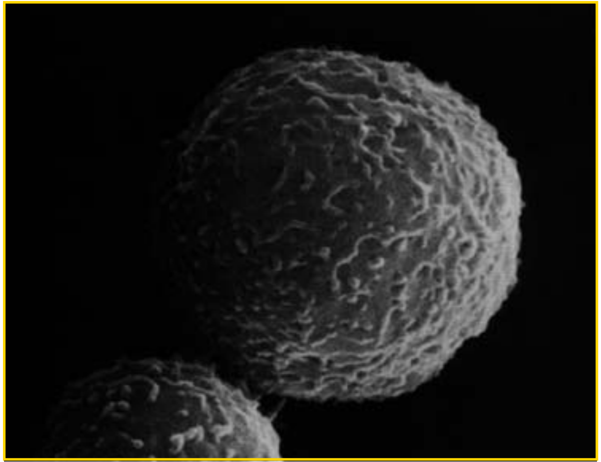
Acknowledgements: TaeYoon Kim, Hyunsuk Lee, Belinda Yap, Jeff Hsu, Jorge Ferrer, Seok Chung

The Neutrophil -- naturally-occurring deformations

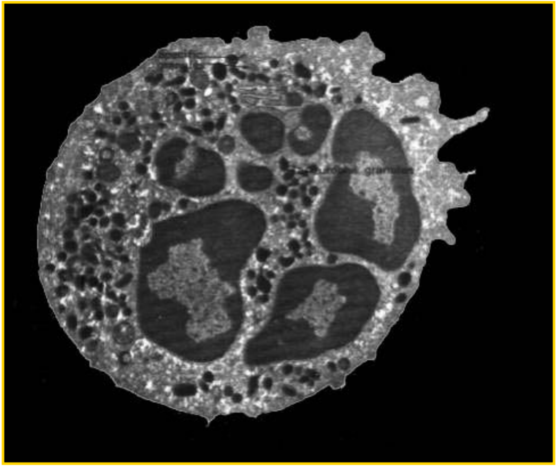
SEM



(micrograph courtesy of C. Dong and R. Skalak)

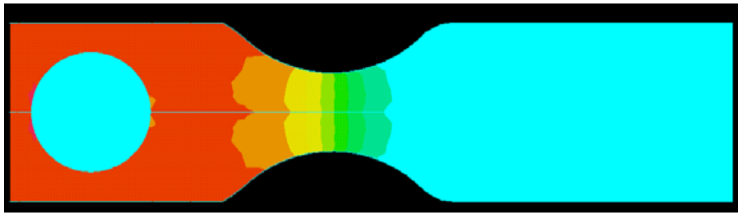
- spherical
- mean diameter = 6.8 µm

Cross-section



(micrograph courtesy of C. Dong and R. Skalak)

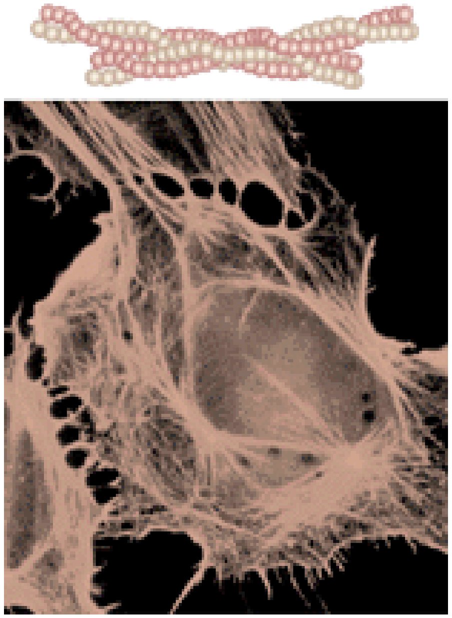
- multi-lobed nucleus
- granules/cytoskeleton
- actin-rich cortical region



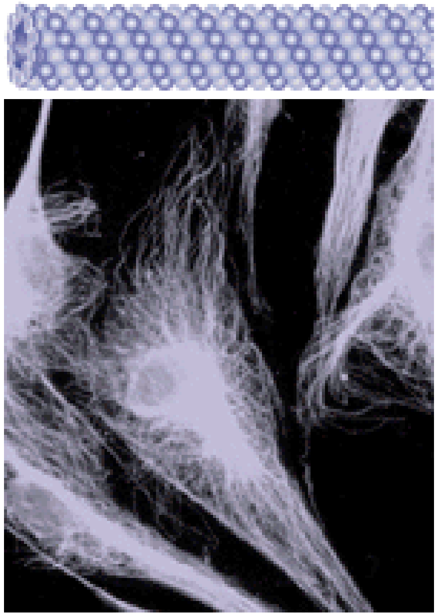
Bathe et al., 2002

Cytoskeletal composition/structure

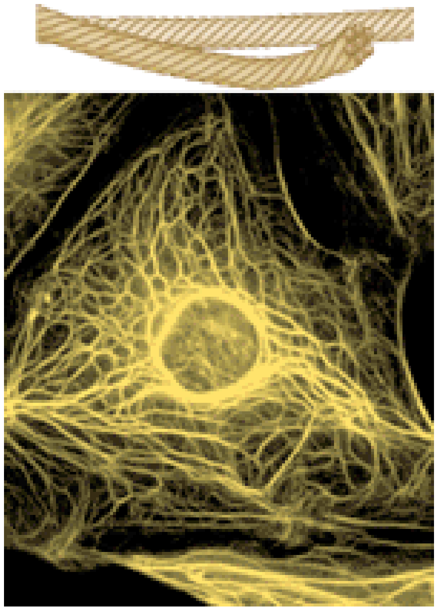
MICROFILAMENTS



MICROTUBULES

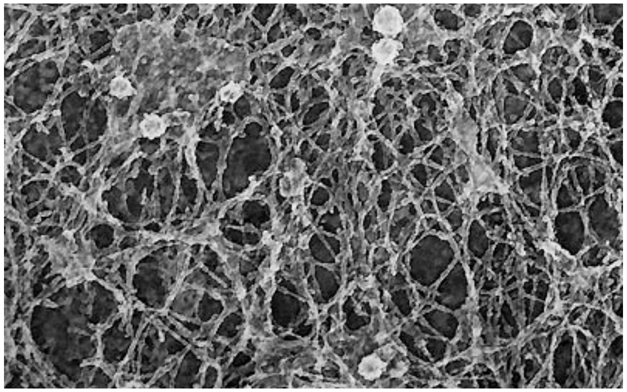


INTERMEDIATE FILAMENTS



Ingber, Scientific American

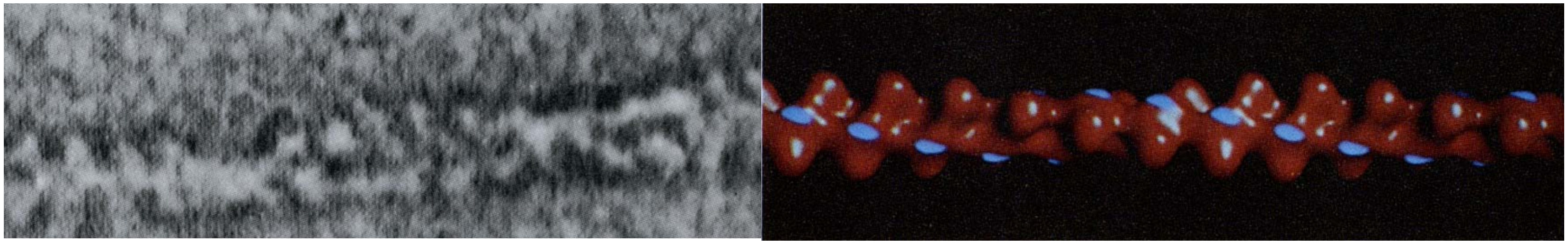
Cells have a multi-component fibrous, gel-like matrix.



Hartwick, http://expmed.bwh.harvard.edu

Actin -- One of the primary structural components of cells

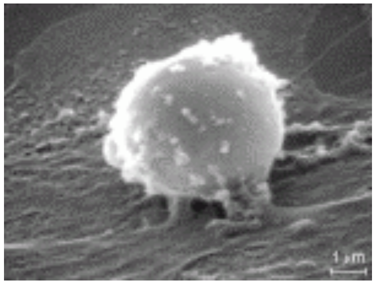
- 40kDa (375 AA residues)
- G-actin to F-actin : Nucleation (lag phase), elongation (growth phase), steady state (equilibrium phase)

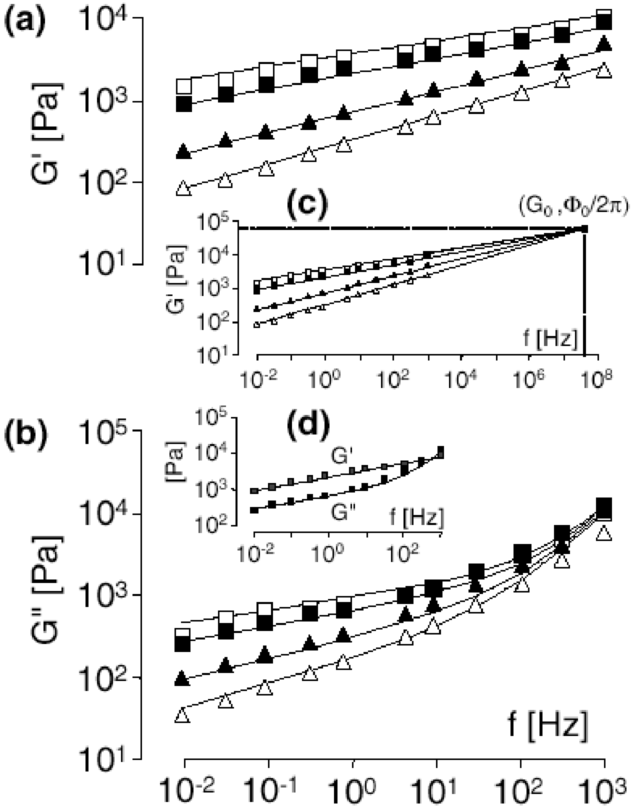


Biochemistry, Wiley 1995

- Semi-flexible filament (neither flexible nor stiff) $l_p \approx l_c$
- Viscoelastic network
- Monomer size << mesh size $\leq l_p$
- Self assembly with polarity
- Binding to a variety of ABPs (Actin Binding Proteins)
- Actin polymerization plays a key role in fundamental cellular processes

Magnetic Twisting Cytometry (MTC)





$$G^* = G' + iG'' = \alpha \frac{\tilde{T}(t)}{\tilde{\delta}(t)}$$

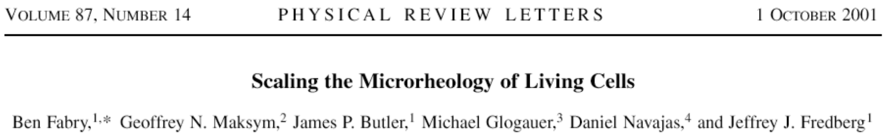
α is a geometry-dependent prefactor determined from finite element analysis

$$G^* = G_0\left(\frac{\omega}{\omega_0}\right)^{x-1}(1+i\bar{\eta})\Gamma(2-x)\cos\left[\frac{\pi}{2}(x-1)\right] + i\omega\mu$$

Γ is the Gamma-function; G_0, Φ_0 and x are adjustable parameters

Consistent with models for soft, glassy materials

Cells exhibit a power-law rheology, reminiscent of a soft , glassy material.

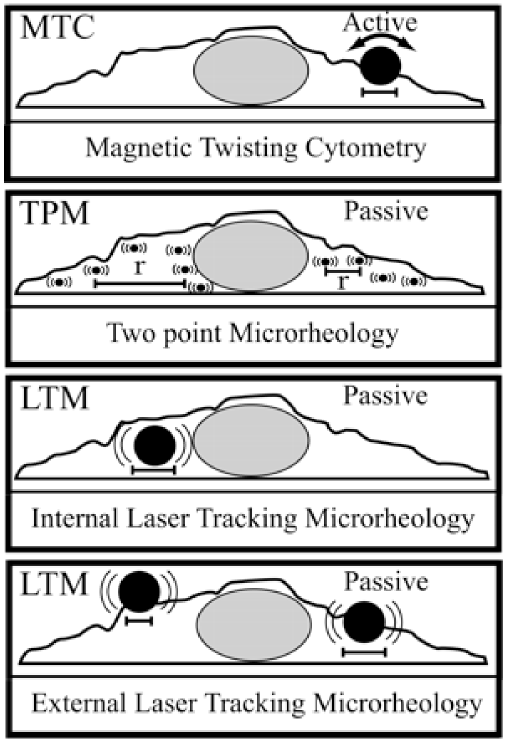


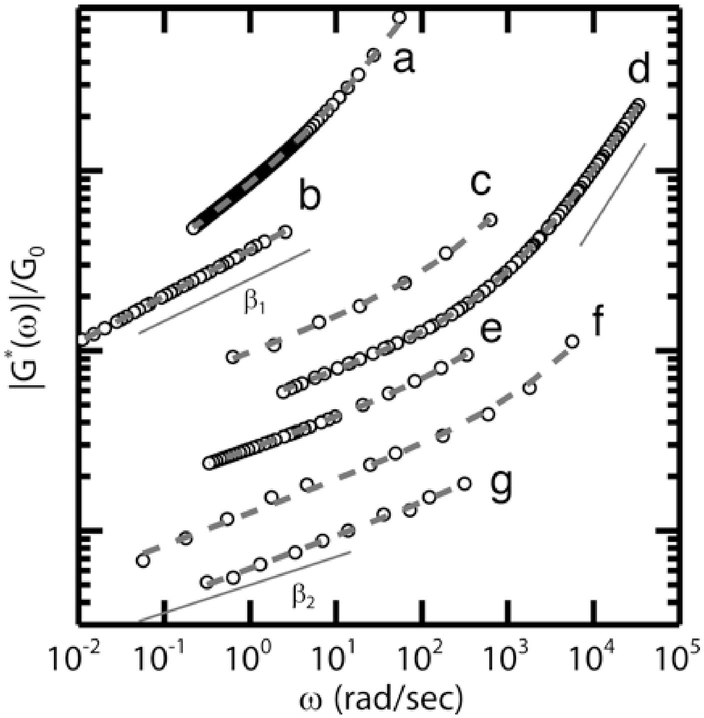
VOLUME 87, NUMBER 14 PHYSICAL REVIEW LETTERS 1 OCTOBER 2001

Scaling the Microrheology of Living Cells

Ben Fabry,[1,*] Geoffrey N. Maksym,[2] James P. Butler,[1] Michael Glogauer,[3] Daniel Navajas,[4] and Jeffrey J. Fredberg[1]

Different behaviors for different types of measurement





Data appear to fall along two characteristic slopes, one for methods that emphasize cortical structure, and another for internal, cytoskeletal structure.

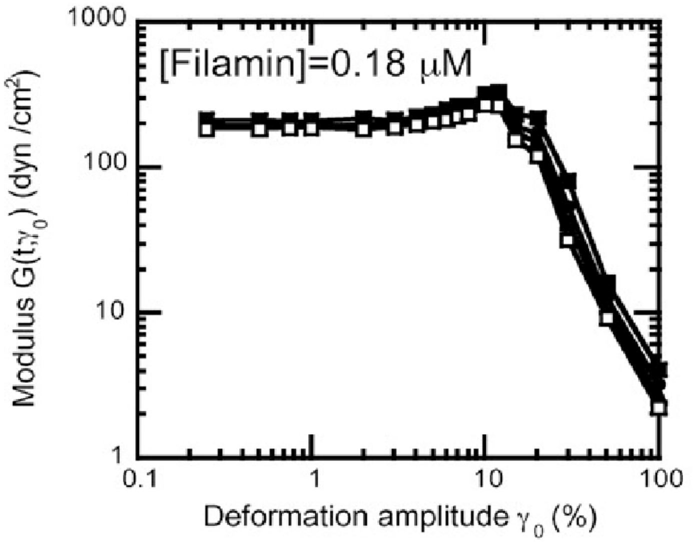
But values for *G* range from 10’s of Pa to several kPa, depending on the method of measurement.

Hoffman, et al., PNAS, 2006

Reconstituted actin gel rheology

Hypotheses for breakdown of the cytoskeleton due to mechanical deformation

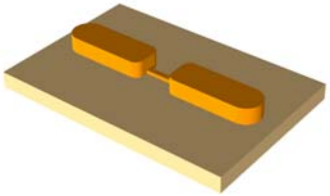
1) Sudden depolymerization of F-actin
2) Rupture of actin cross-links between F-actin filaments

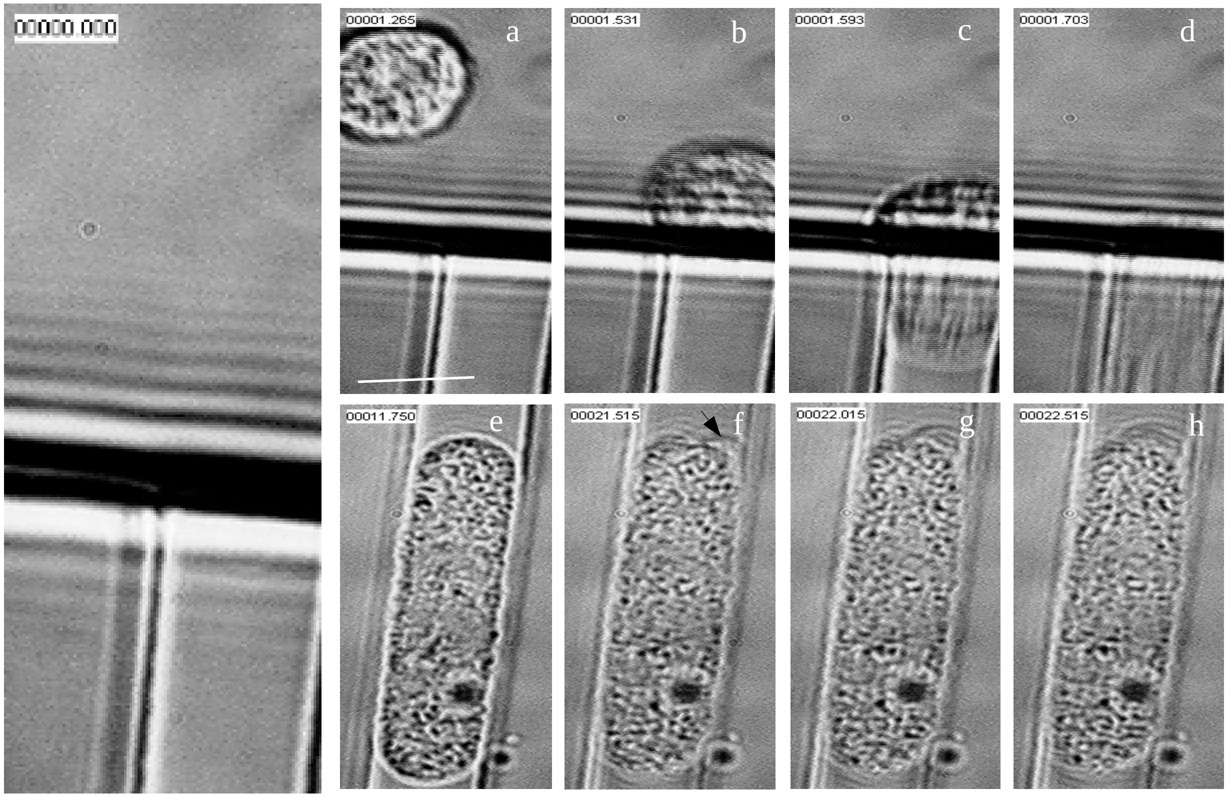


Tseng et. al. (2004) J Biol Chem; 279: 1819-1826

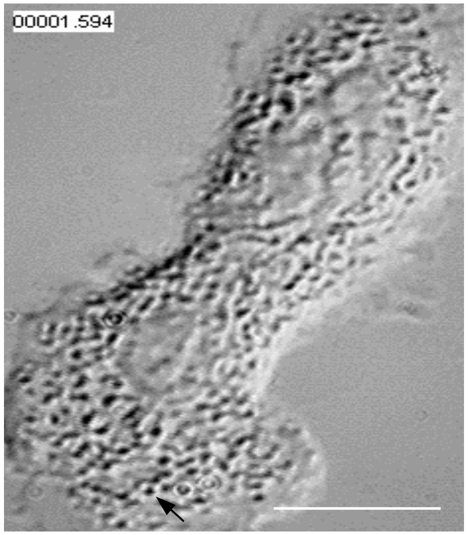
Incremental shear moduli exhibit strain hardening up to a point, followed by a catastrophic drop in stiffness.

Cytoskeletal shear modulus computed from the Brownian fluctuations of granules

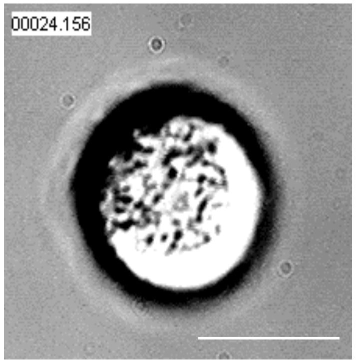




As a function of time after entering channel, until protrusion

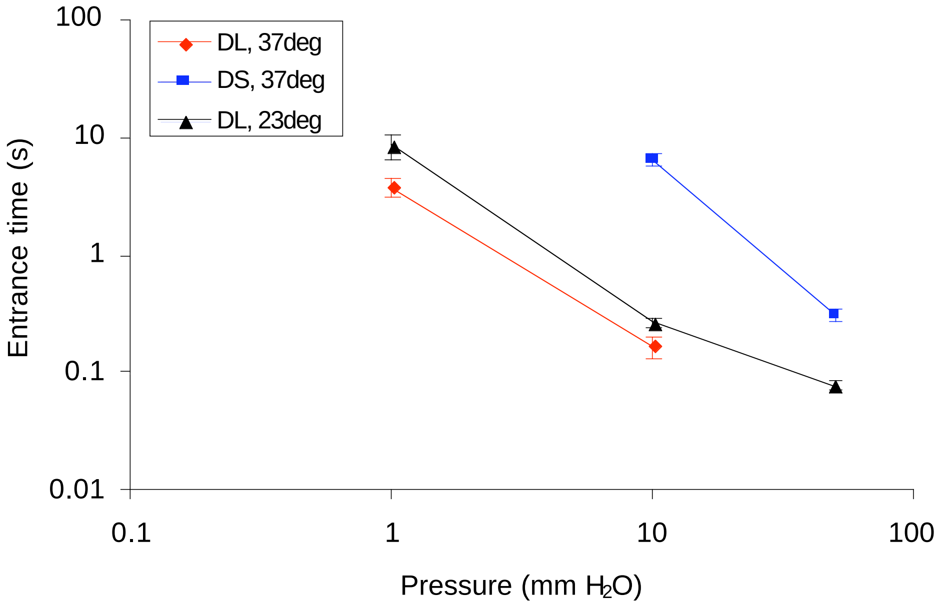


In adherent, migrating neutrophils

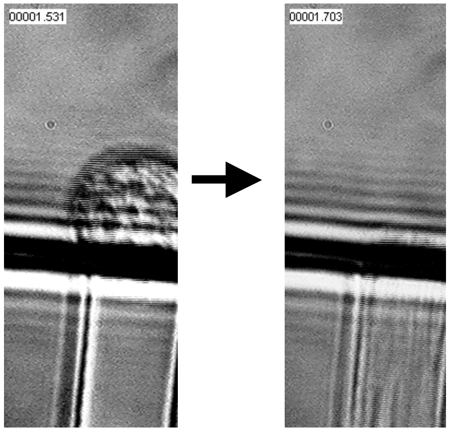


In non-adherent, neutrophils

Entrance Time -- Passive Behavior



Entrance time appears primarily influenced by the magnitude of mechanical stimulation; less so by biological activity

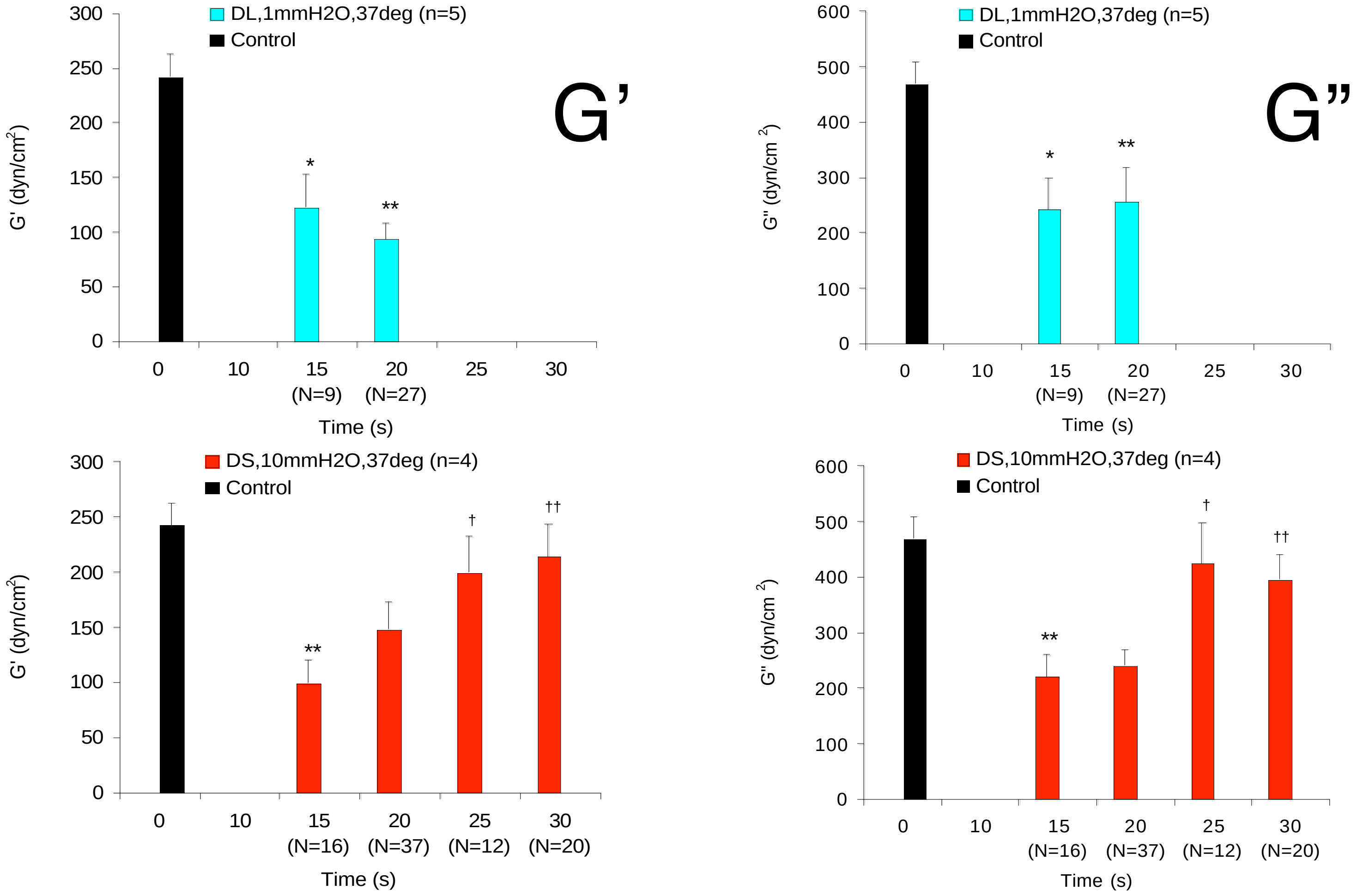


Entrance time : interval between leading edge touching channel entrance and trailing edge clearing channel entrance after deformation

To what extent can the cytoskeleton be treated as a cross-linked actin gel?

Yap & Kamm, APS, 2005

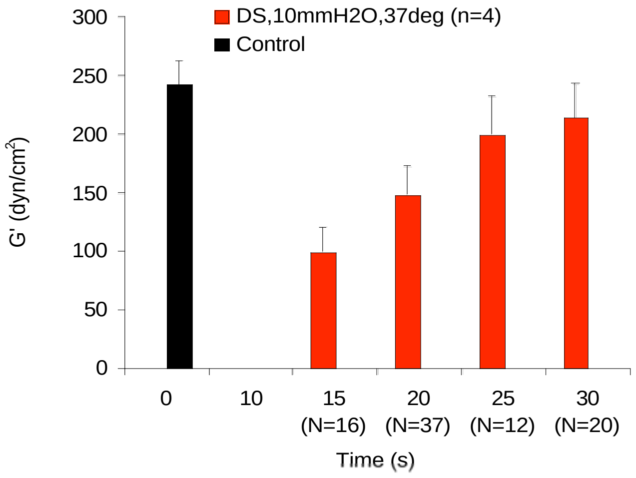
Viscoelastic moduli of neutrophils following deformation



Moduli fall immediately upon deformation, and recover on a time scale of about one minute

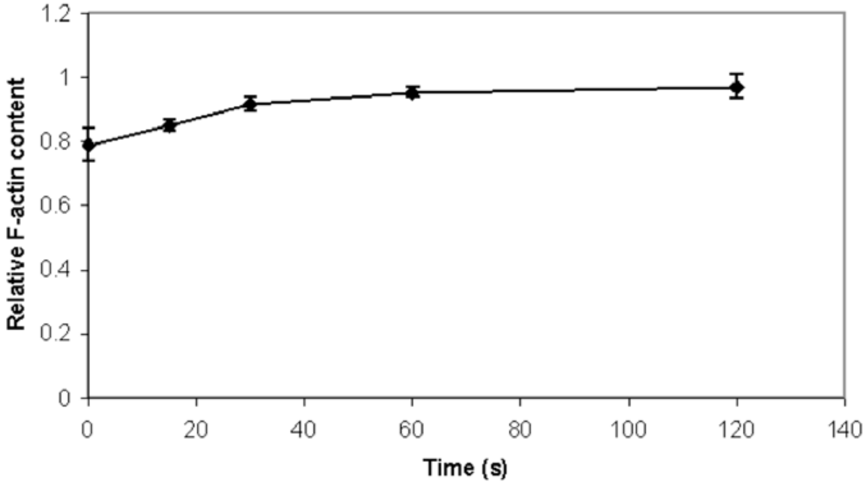
Dynamic shear modulus and F-actin follow similar patterns

Storage modulus drops immediately, then recovers on a time scale of 30-60s.



Resting neutrophils

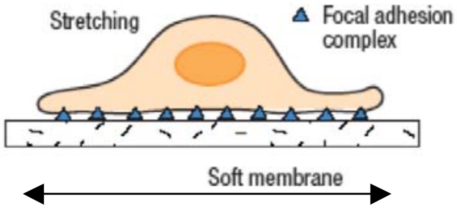
Post-deformation

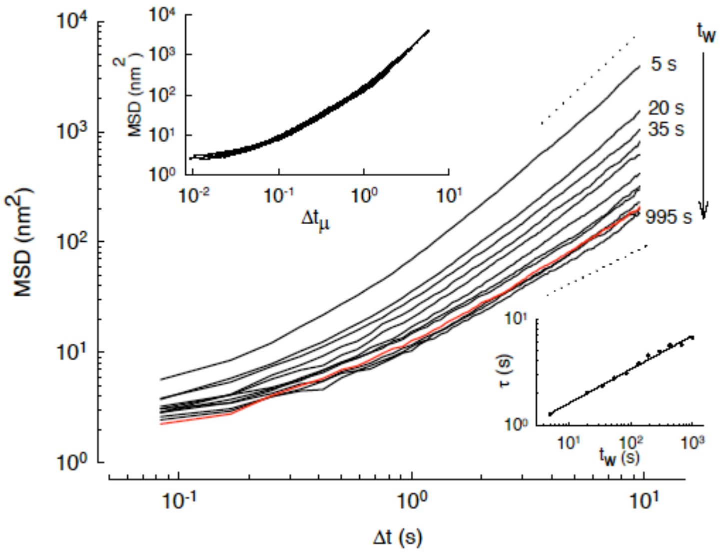


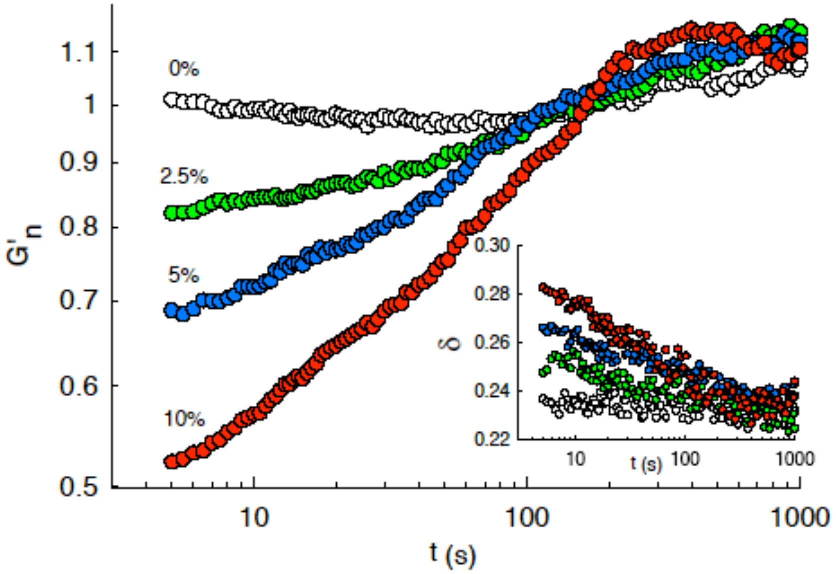
A similar time-course is observed for F-actin content following passage through pores.

Actin depolymerization appears to explain part, but perhaps not all, of the drop in modulus

Similar behavior -- abrupt fall, then recovery of stiffness -- has been observed in other cell types







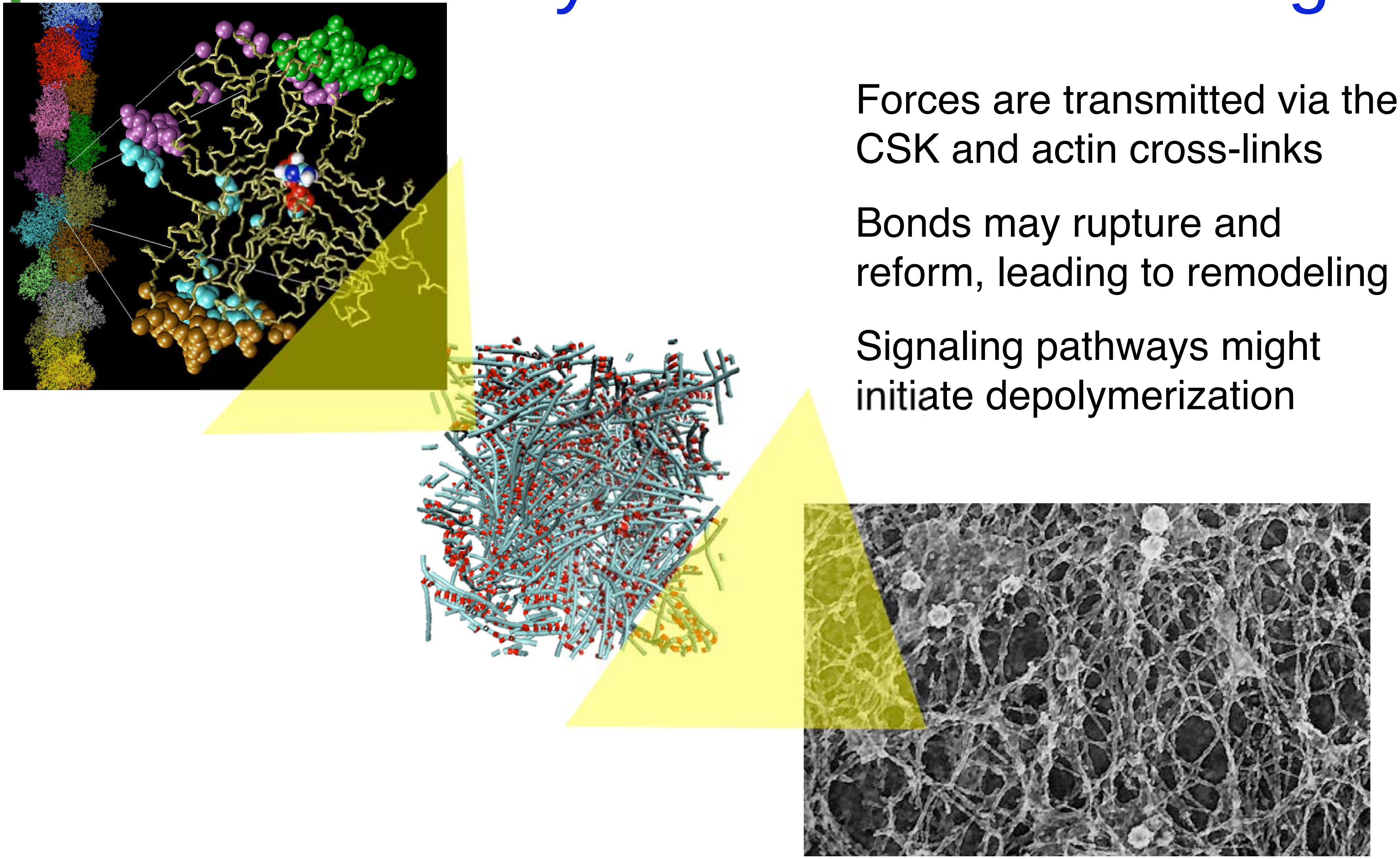
Universal physical responses to stretch in the living cell

10 November 2006

Xavier Trepat[1], Linhong Deng[1,2], Steven S. An[1,3], Daniel Navajas[4], Daniel J. Tschumperlin[1], William T. Gerthoffer[5], James P. Butler[1], and Jeffrey J. Fredberg[1]

Cytoskeletal fluidization is a common attribute of cells

Cytoskeletal remodeling



Forces are transmitted via the CSK and actin cross-links

Bonds may rupture and reform, leading to remodeling

Signaling pathways might initiate depolymerization

Some outstanding questions

What is the physical basis for soft, glassy rheology?

How can strain stiffening in reconstituted actin gels be reconciled with fluidization in cells under abrupt strains?

Are the differences in behavior seen with different measurement methods real, and how can they be reconciled?

Are the different power law behaviors really due to cortex vs. cytoskeleton? If so, why?

Modeling Objectives

Simulate actin cytoskeletal growth, rheology, and force-induced changes in biochemical activity using molecular dynamics, Brownian dynamics, and continuum models.

Cytoskeleton Growth

- Investigate the effects and roles of the properties of actin cross-linking proteins (ACPs) on cytoskeletal structure

Microbead Rheology

- Estimate viscoelasticity of the actin cytoskeleton using microbead tracking rheology, both active and passive.
- Investigate the effect of ACPs on viscoelasticity

Relevant recent works: Storm, et al., Nature (London), 2005
Hoffman, et al., http://arxiv.org/pdf/physics/0504051

Cytoskeletal Modeling

Equation of motion: Langevin equation

$$m\frac{d^2\vec{r}}{dt^2}=\sum_{i\neq j}\vec{f}_{i\,j}-\zeta\frac{d\vec{r}}{dt}+\vec{d}(t)$$

Drag computed (initially) ignoring hydrodynamic interactions

Integration Method: Euler forward (dropping inertia)

$$\frac{d\vec{r}^*}{dt^*}=\sum_{i\neq j}\vec{f}^*_{i\,j}+\vec{d}^*(t)\quad\rightarrow\quad d\vec{r}^*=\left[\sum_{i\neq j}\vec{f}^*_{i\,j}+\vec{d}^*(t)\right]dt^*$$

Interaction forces:

Truncated Lennard-Jones

$$U_{LJ}(r)=\begin{cases}4\varepsilon\left[\left(\dfrac{\sigma}{r}\right)^{12}-\left(\dfrac{\sigma}{r}\right)^{6}\right]+\varepsilon & r\leq 2^{1/6}\sigma\\ 0 & r>2^{1/6}\sigma\end{cases}$$

Bending stiffness

$$U_{Bend}=\frac{1}{2}\kappa_{Bend}\left(\theta-\theta_0\right)^2$$

Extensional stiffness

$$U_{Spring}=-\frac{1}{2}k_s(r-r_0)^2$$

Torsional stiffness

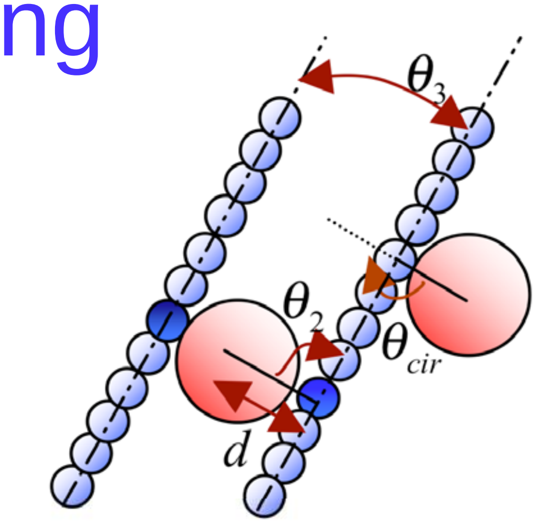
$$U_{Torsion}=\frac{1}{2}\kappa_{Torsion}\left(\theta-\theta_0\right)^2$$

Scalings:

Length scaled with actin radius, energy with k_BT

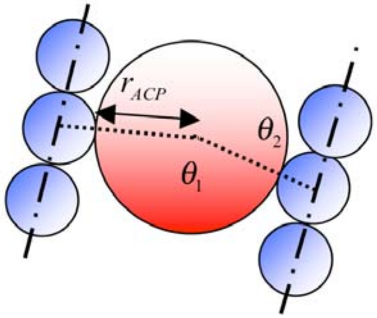
$$t^*=\frac{t}{\sigma^2\zeta/k_BT}$$

Cross-linking



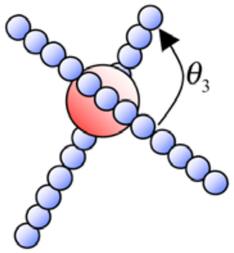
Unbinding obeys Bell’s equation and is force-dependent.

Bundling cross-linkers (e.g., fimbrin, fascin, scruin, α-actinin)



$$r_{ACP} = 0.75,\ \theta_1 = \pi,\ \theta_2 = \frac{\pi}{2},\ \theta_3 = 0$$

Network cross-linkers (e.g., filamin)

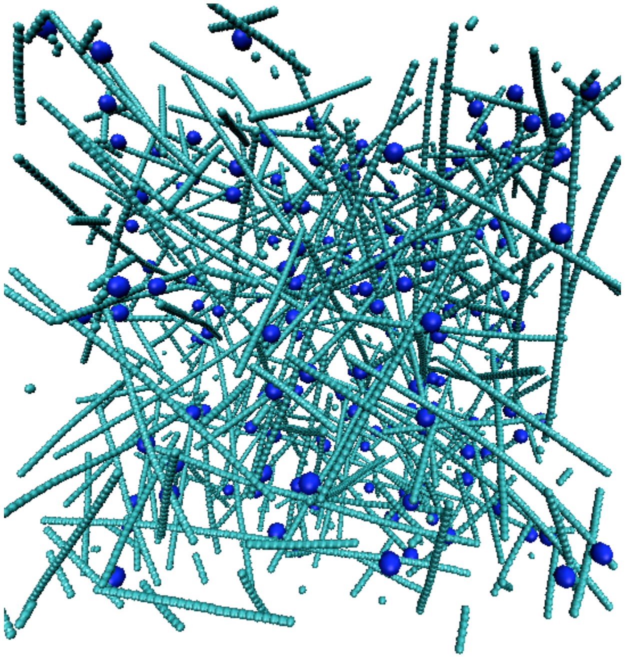


$$r_{ACP} = 1.5,\ \theta_1 = 1.158\,(rad),\ \theta_2 = \frac{\pi}{2},\ \theta_3 = \frac{\pi}{2}$$

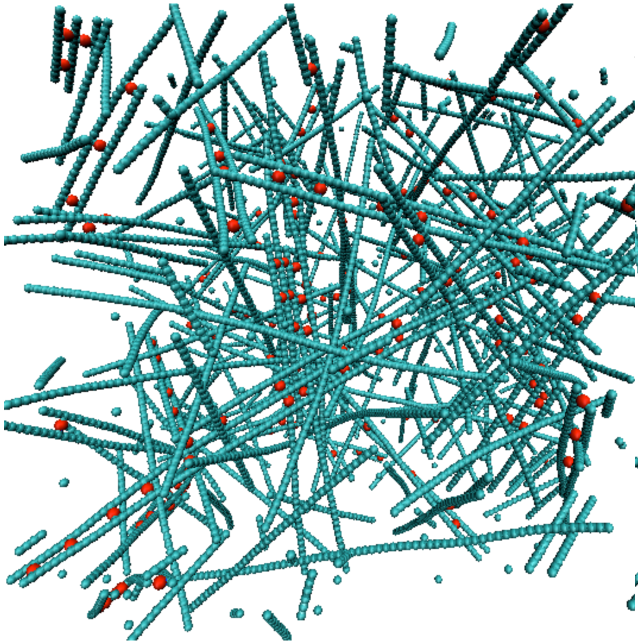
Comparison of cross-linked and bundled structures

(varied by changing the torsional stiffness and orientation of actin binding sites)

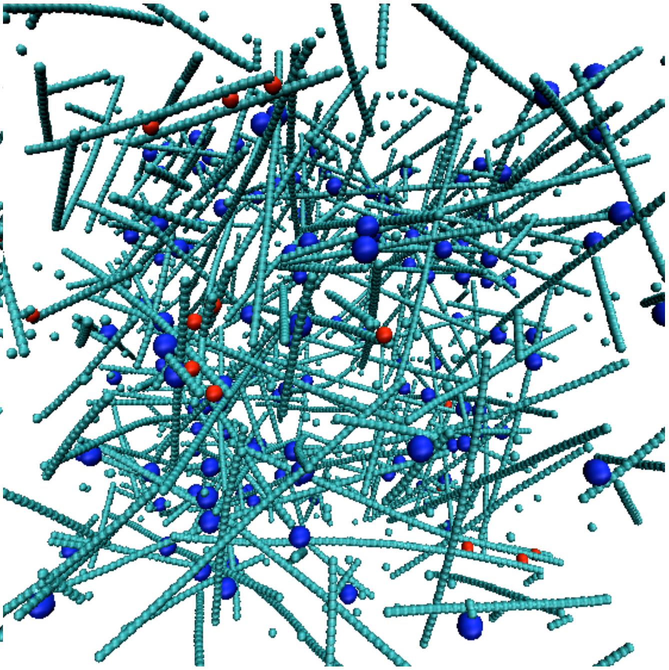
Cross-linked



Bundled



Mixed

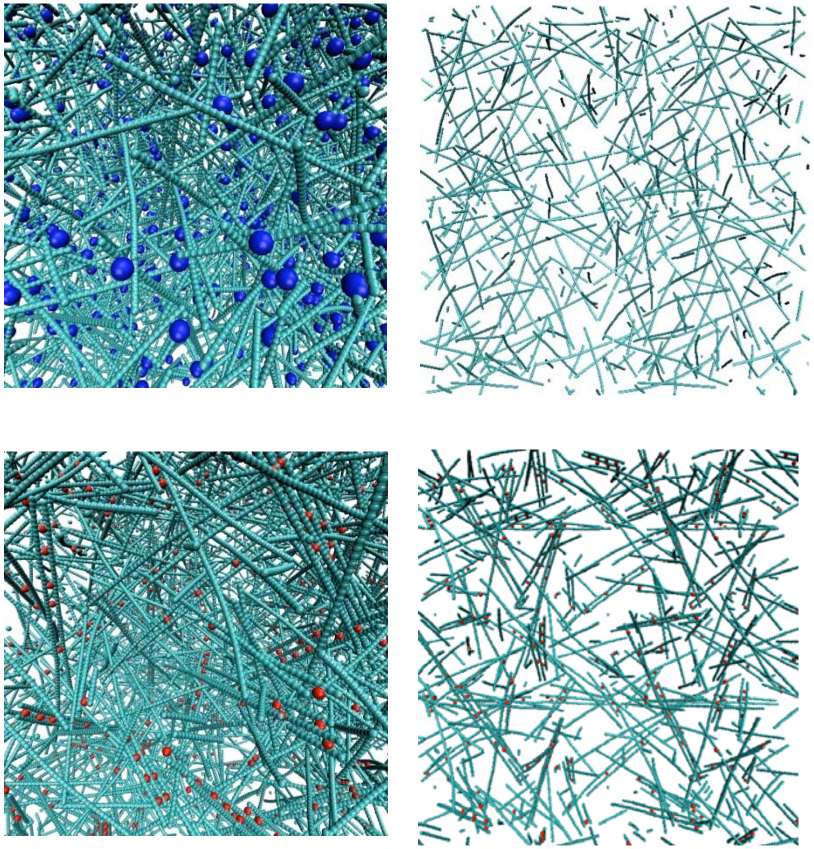


Monomers : 8,000, ACPs : 1,000
Vol. % = 0.82% (+ACP ~0.1%), R=0.125
Conc = 150 μM, Domain = 560 nm

Computed Network Structures

90° cross-links

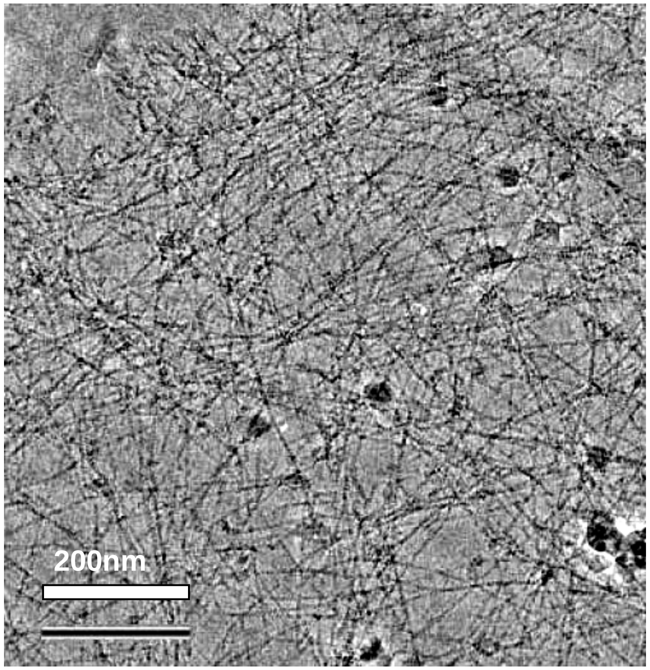
“Bundling” cross-links



Computed 3D images

“Confocal” images

1.12 µm x 1.12 µm

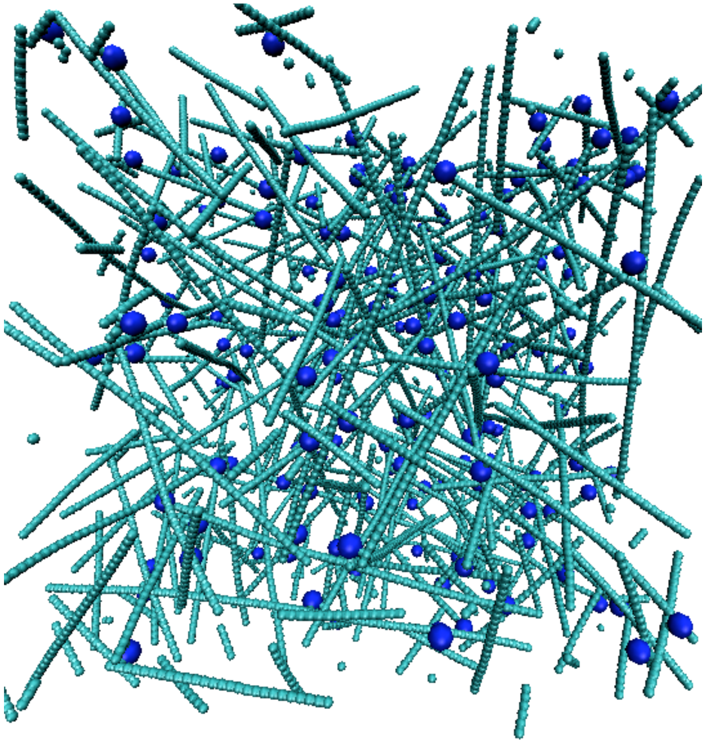


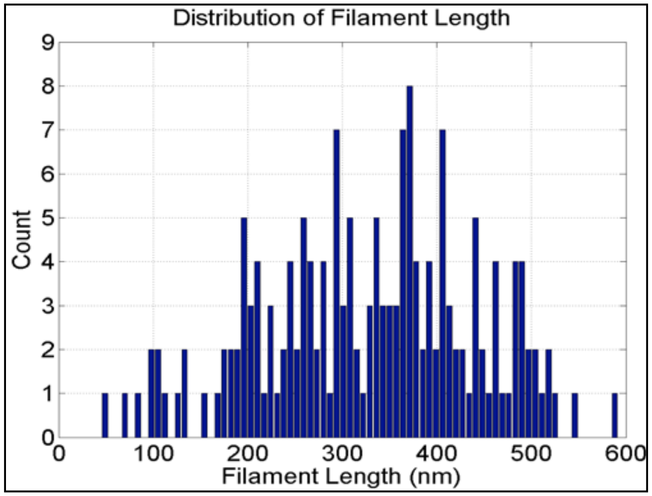
Visualisation of the actin cytoskeleton by cryo-electron microscopy

Guenter P. Resch[1], Kenneth N. Goldie[2], Angelika Krebs[2], Andreas Hoenger[2] and J. Victor Small[1,*]

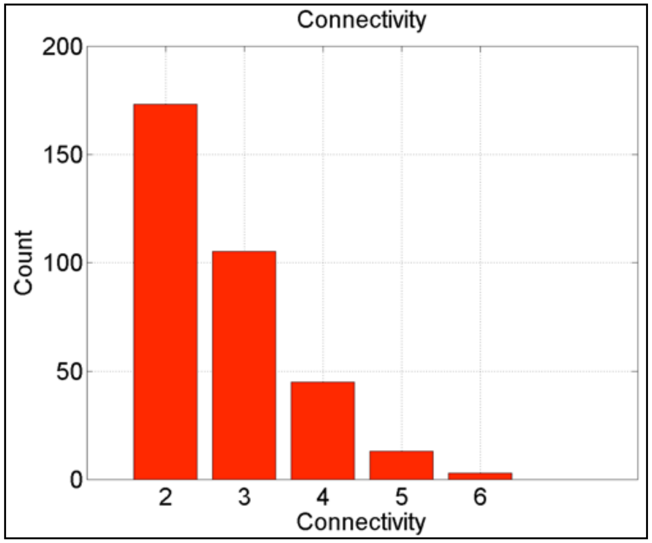
Journal of Cell Science 115 (9)

Analysis of the Polymerized Structure





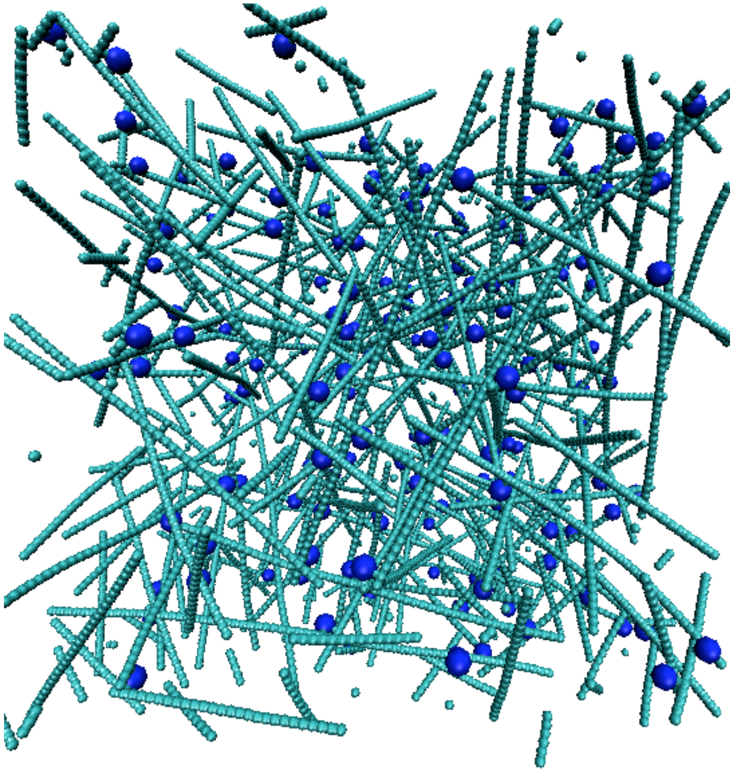
Avg = 328, S.D. = 112

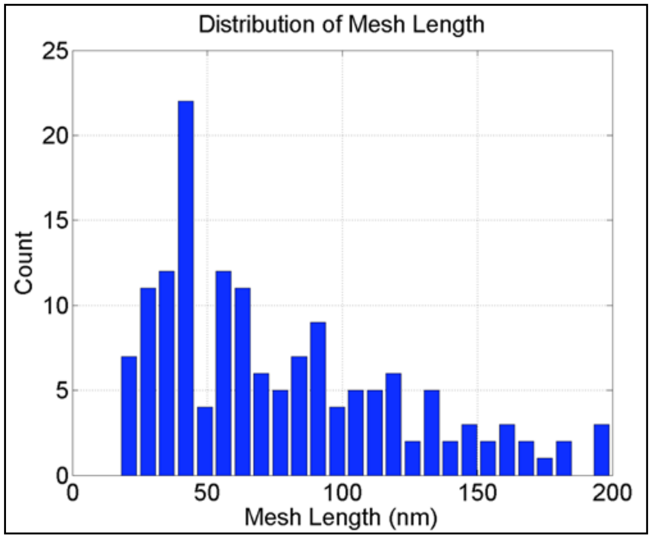


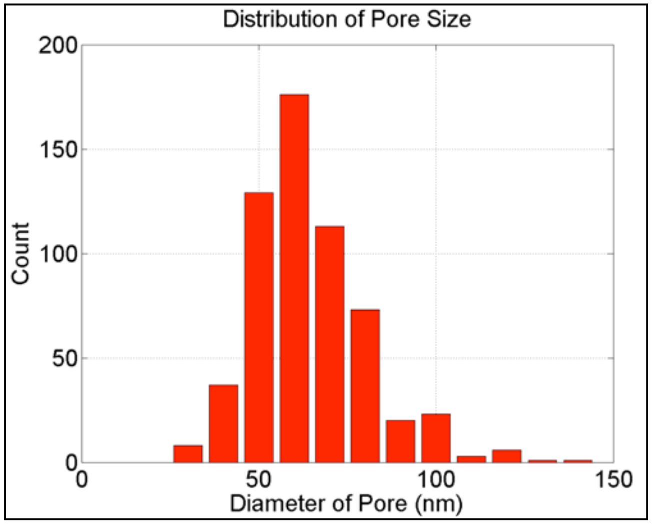
Monomers : 8000, ACPs : 173
Vol. % = 0.82% = 150 μM
Nucleation rate = 1 x 10^{-8} s^{-1}, Domain = 560 nm

Filament length = total contour length
Connectivity = number of filaments attached
via cross-links

Analysis of the Polymerized Structure





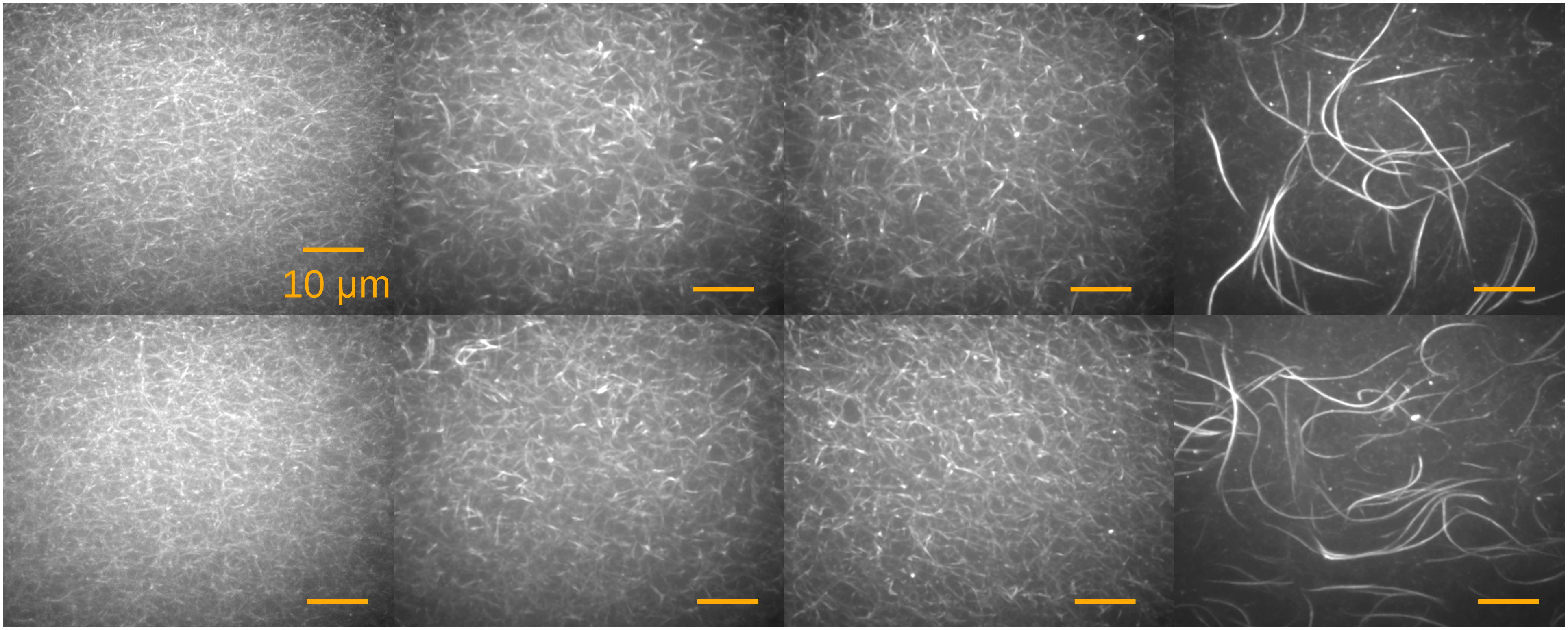


Monomers : 8000, ACPs : 173
Vol. % = 0.82% = 150 μM
Nucleation rate = 1 x 10^{-8} s^{-1}, Domain = 560 nm

Mesh size = distance between cross-links
Pore size = max diameter of a sphere that can fit inside a pore

F-actin networks formed by α-actinin

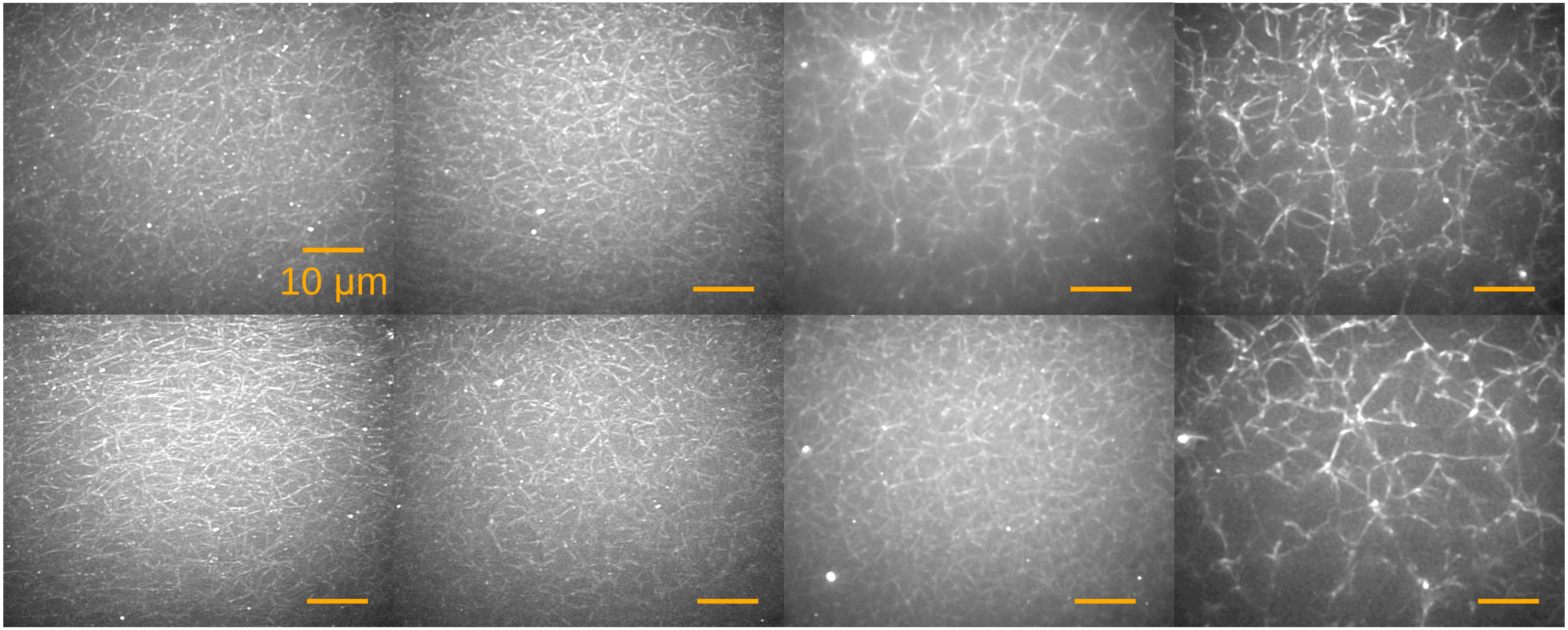
R_α = 0.01 R_α = 0.1 R_α = 0.2 R_α = 0.5

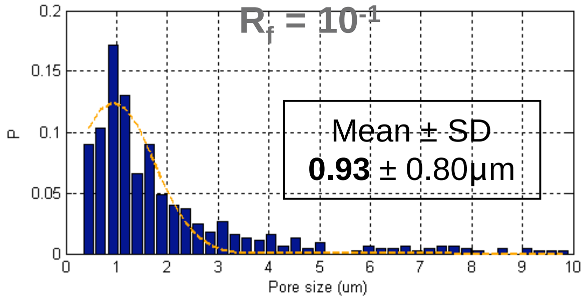


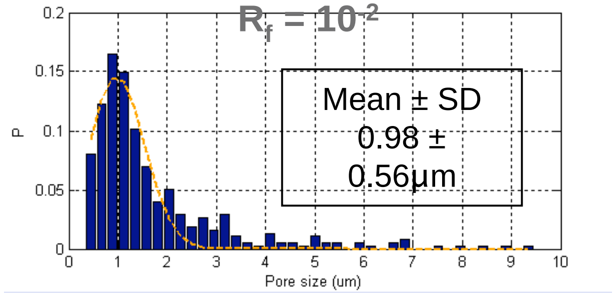
Stress fibers form of increasing diameter as the amount of α-actinin is increased

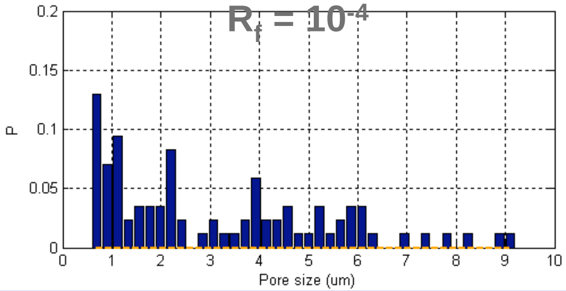
F-actin networks formed by filamin

$R_f = 10^{-1}$ $R_f = 10^{-2}$ $R_f = 10^{-3}$ $R_f = 10^{-4}$





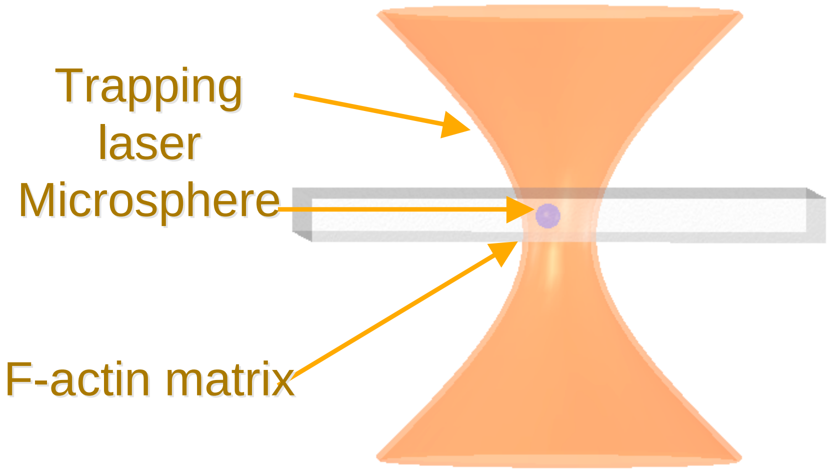




Passive microrheology

Monitor thermal fluctuations of embedded microspheres to estimate the frequency dependent complex modulus

High spatial and temporal resolution using quadrant photo detector



Thermally driven dynamics of the microsphere

Fluctuation-dissipation theorem

Kramers-Kronig relation

Generalized Stokes-Einstein relation

$$\left|G^*(\omega)\right| \approx \frac{k_B T}{\pi a \left\langle \Delta r^2(1/\omega) \right\rangle \Gamma\left[1+\alpha(\omega)\right]}$$

$$G'(\omega) = \left|G^*(\omega)\right| \cos(\pi\alpha(\omega)/2)$$

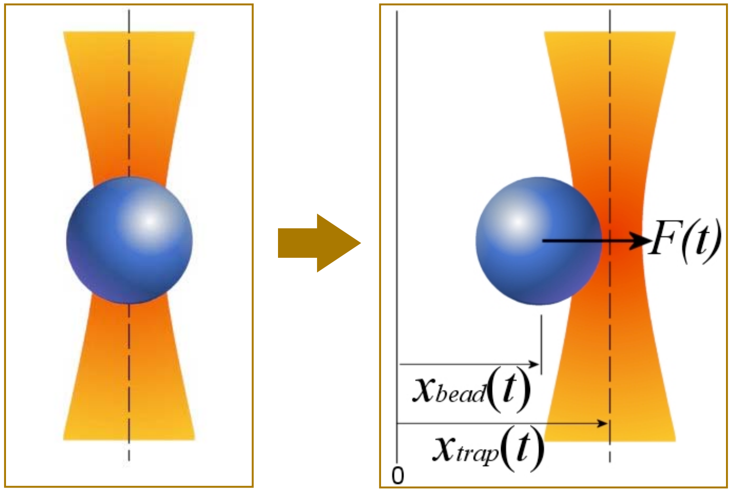
$$G''(\omega) = \left|G^*(\omega)\right| \sin(\pi\alpha(\omega)/2)$$

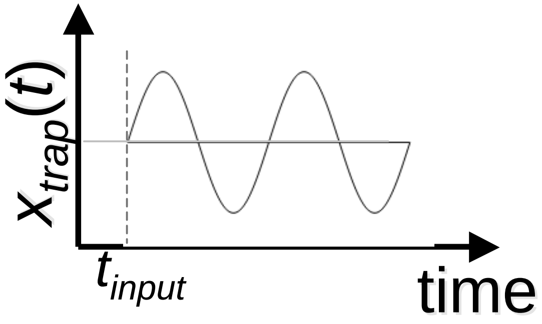
$$\alpha(\omega) \equiv \left.\frac{d\ln\left\langle \Delta r^2(t) \right\rangle}{d\ln t}\right|_{t=1/\omega}$$

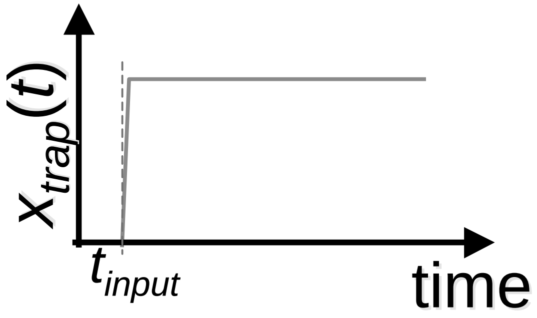
Active microrheology

Apply a driving force to an embedded microsphere and monitor its trajectory

- Sinusoidal and stepwise



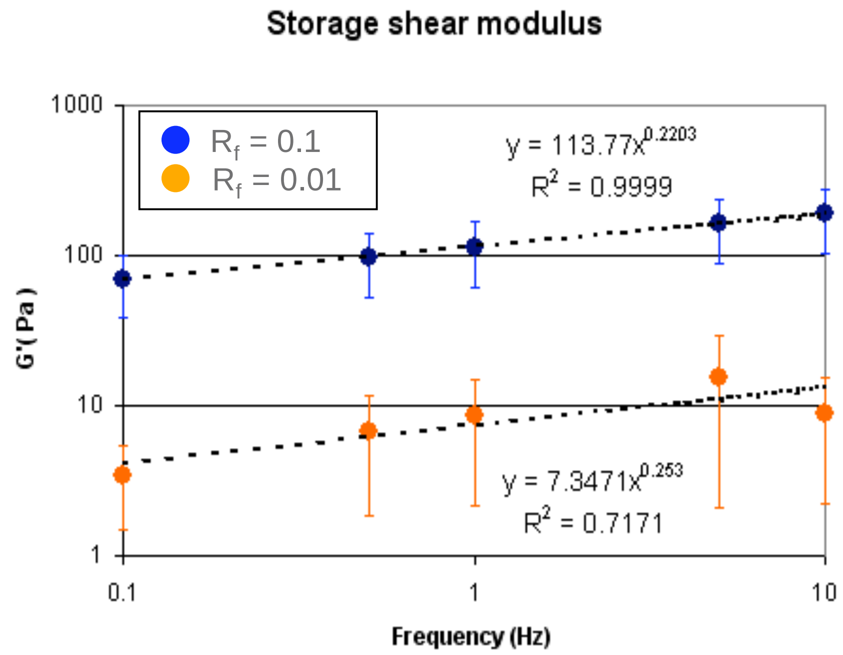


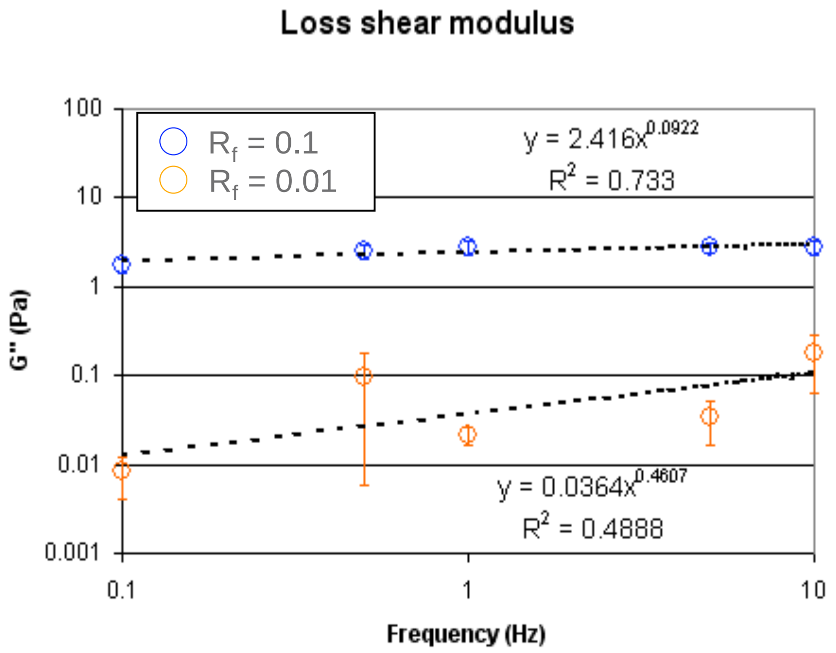


Bulk rheometer

- Parallel plate, AR2000, *TA Instruments*
- Gap: 120µm – 40mm flat plate
- Frequency sweep

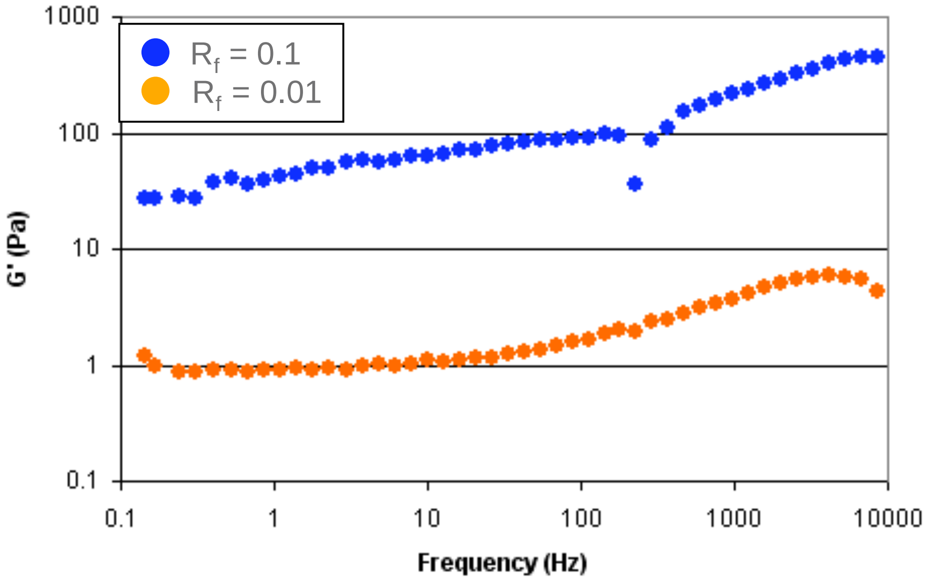
Comparison at different filamin concentrations – Active sinusoidal forcing


Storage shear modulus

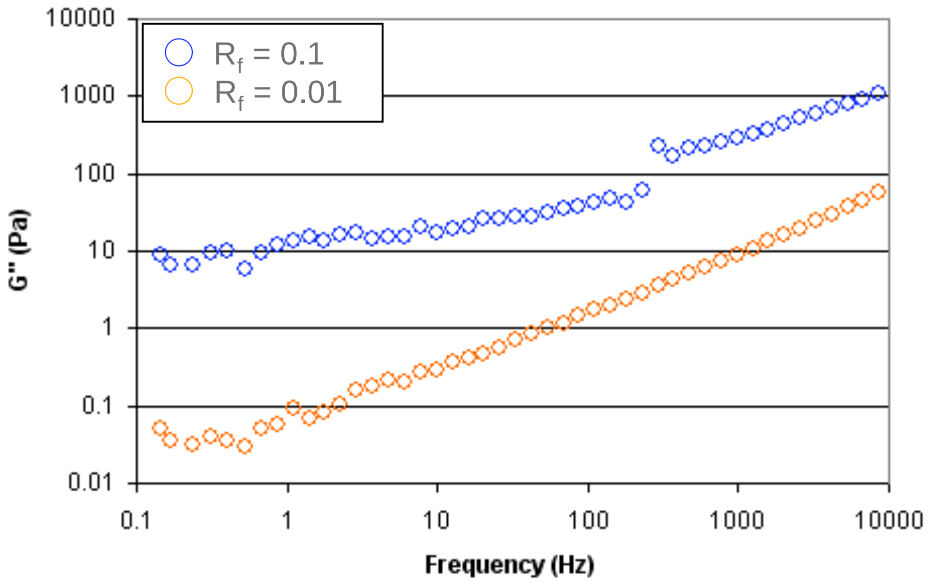

Loss shear modulus

- One order magnitude difference of moduli as R_f increases 10 fold
- $G'_a \sim R_f^{0.2}$ for 10 µM actin, $R_f = 0.01$ and 0.1
- G'_a of $R_f = 0.1$ is comparable to cellular rheology

Comparison at different filamin concentrations – Passive measurement



Storage shear modulus

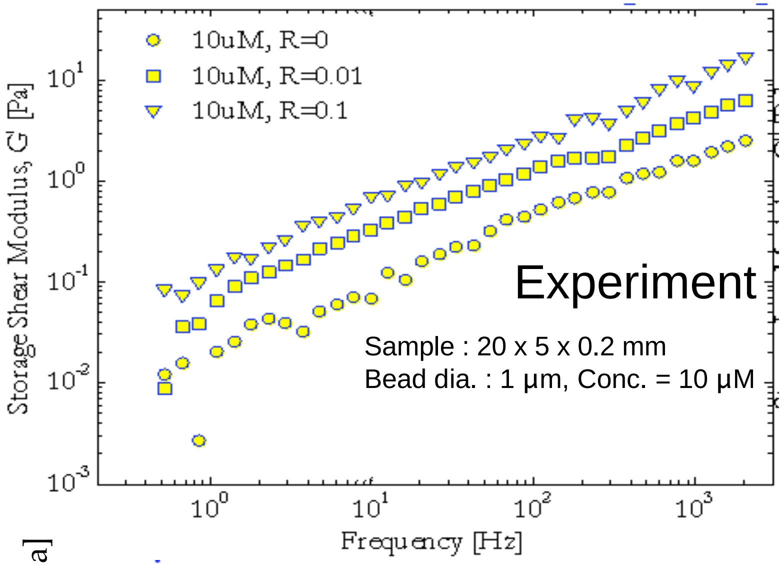


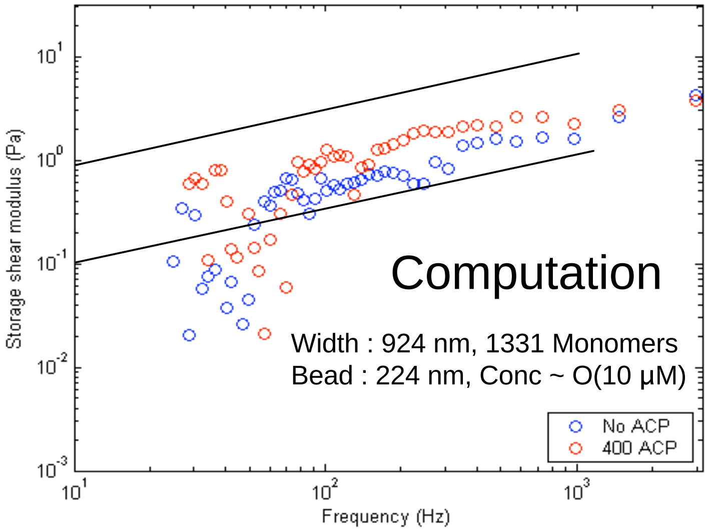
Loss shear modulus

- Longer plateau region in G'_p of $R_f = 0.01$
- Similar values of G'_p in low frequency region

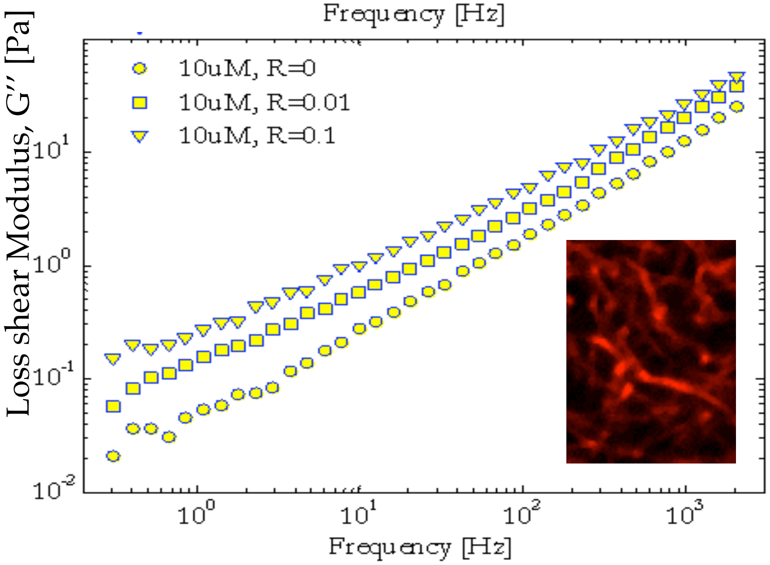
Comparison of complex moduli

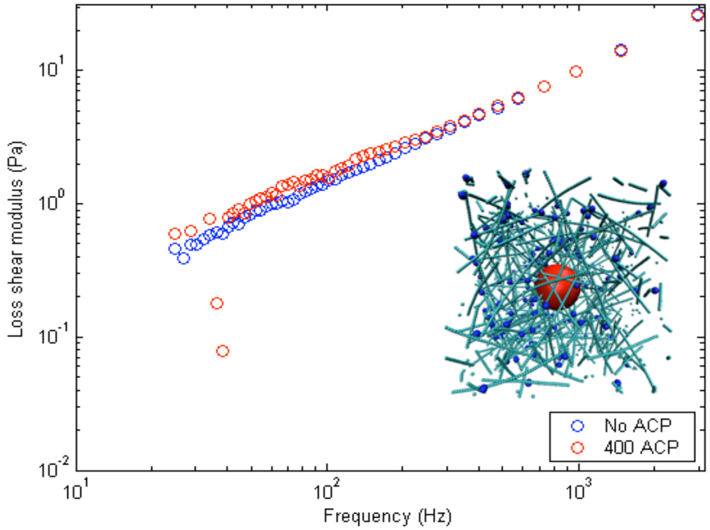
G’





G’’





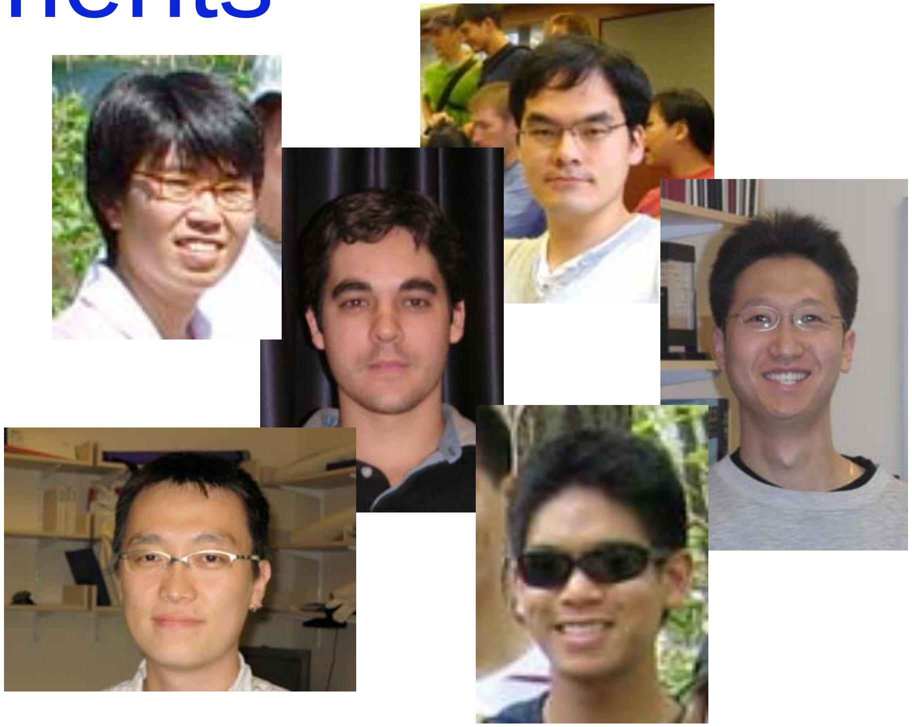
Summary and remaining questions

- Simulated networks exhibit structures and rheology "similar" to those found in experiments in cross-linked gels.

- Can a computational model be created that replicates cellular measurements?
- What is the fundamental basis for cytoskeletal fluidization, correct power-law scaling?
- What is the role of measurement method (1- vs. 2-particle methods, active vs. passive, bead coating, particle size vs. mesh size) in cell rheological measurements?

"If we knew what it was we were doing, it would not be called research, would it?" - Albert Einstein

Acknowledgements



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