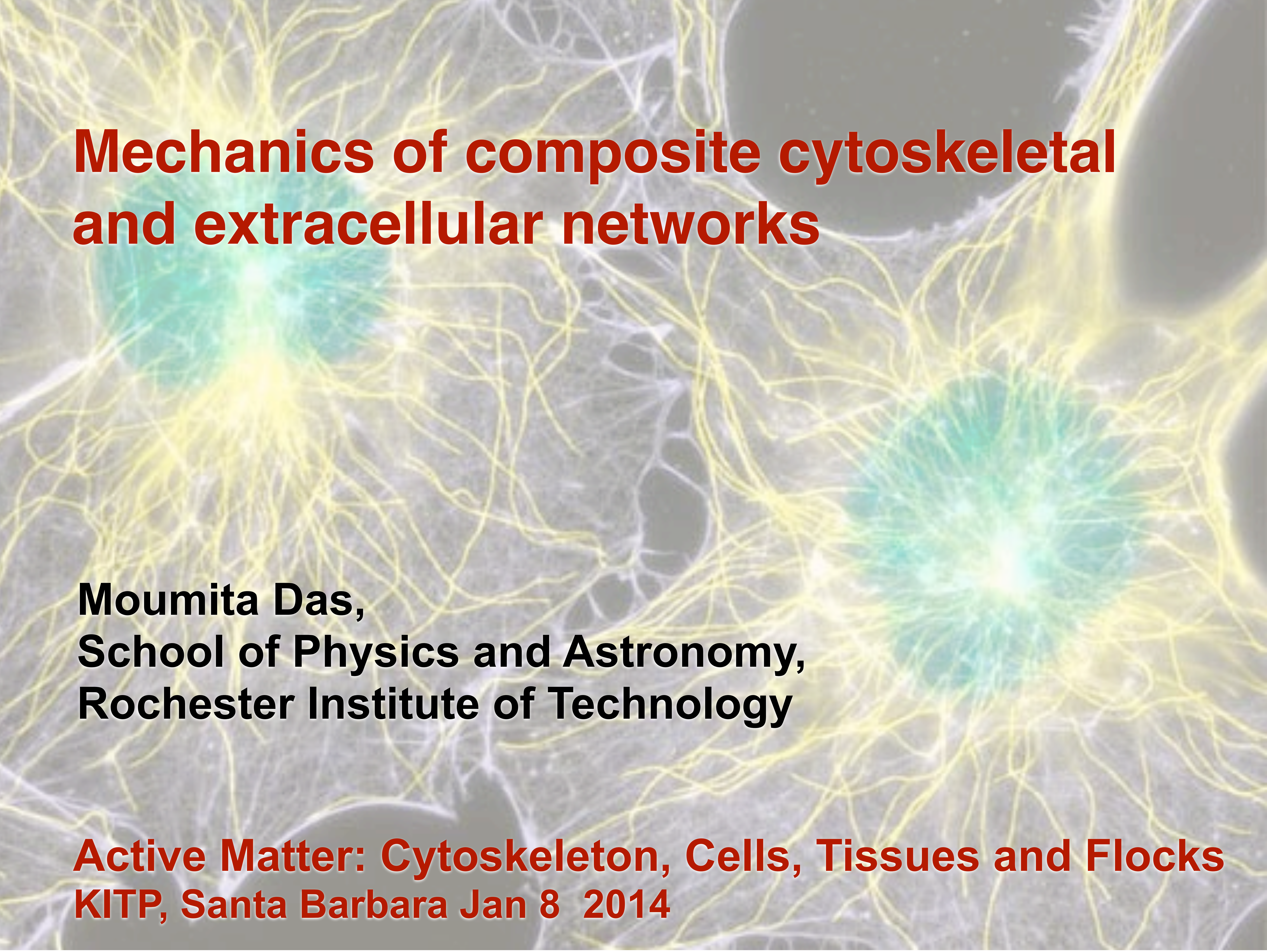


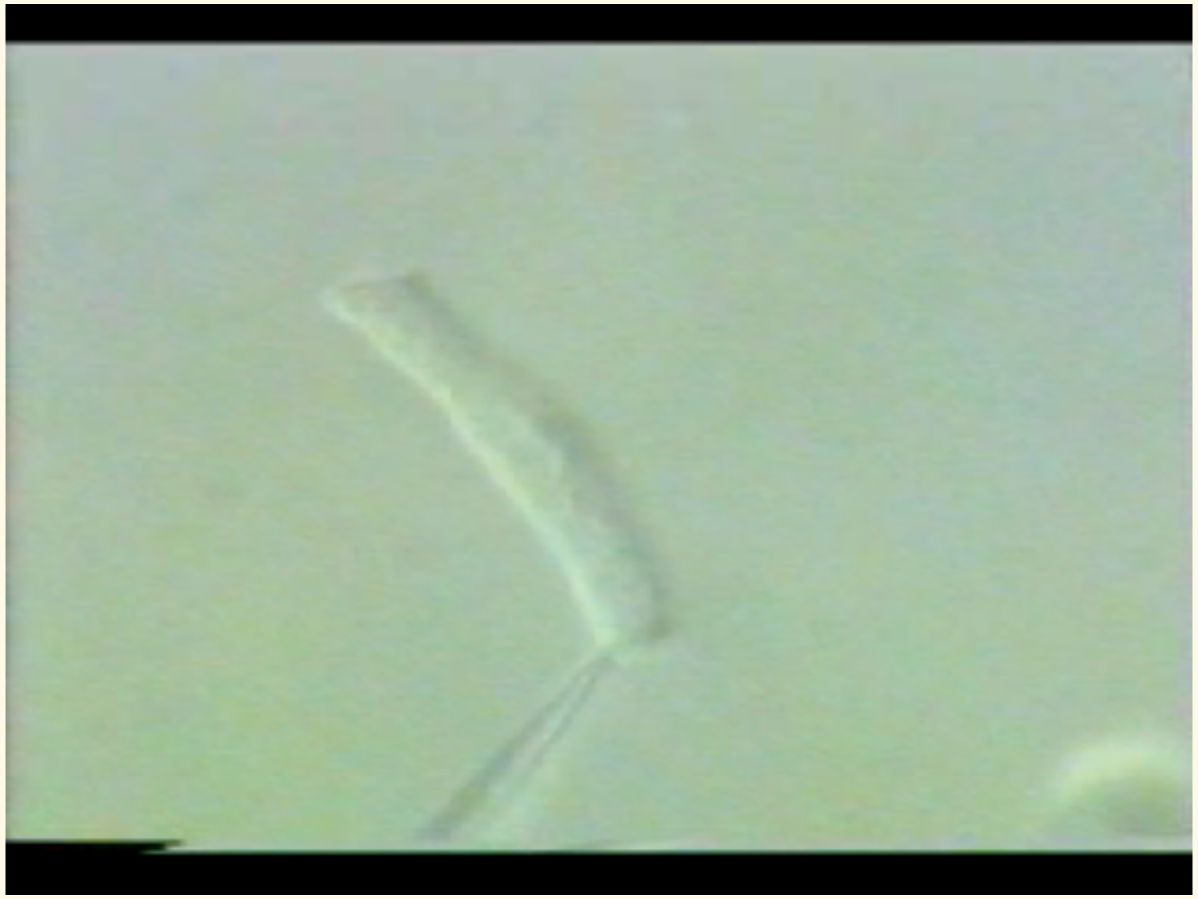
Mechanics of composite cytoskeletal and extracellular networks

Moumita Das,
School of Physics and Astronomy,
Rochester Institute of Technology

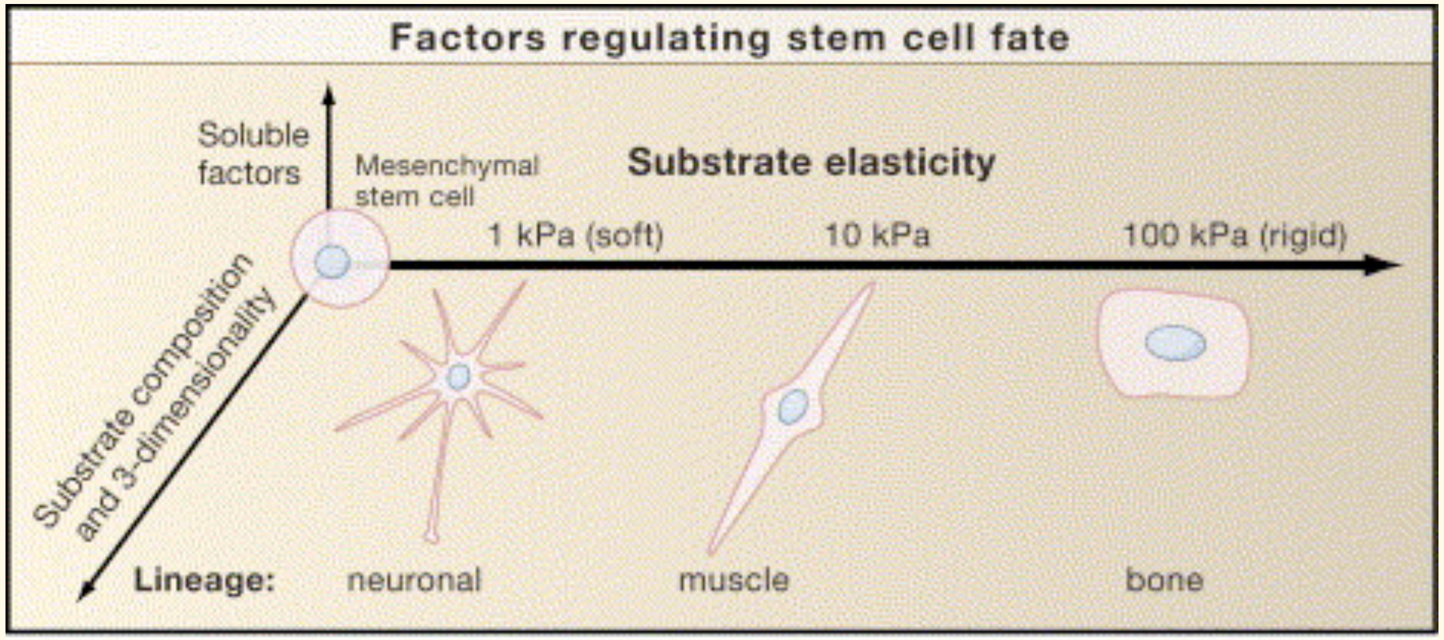
Active Matter: Cytoskeleton, Cells, Tissues and Flocks
KITP, Santa Barbara Jan 8 2014

Cell mechanics modulates function and fate

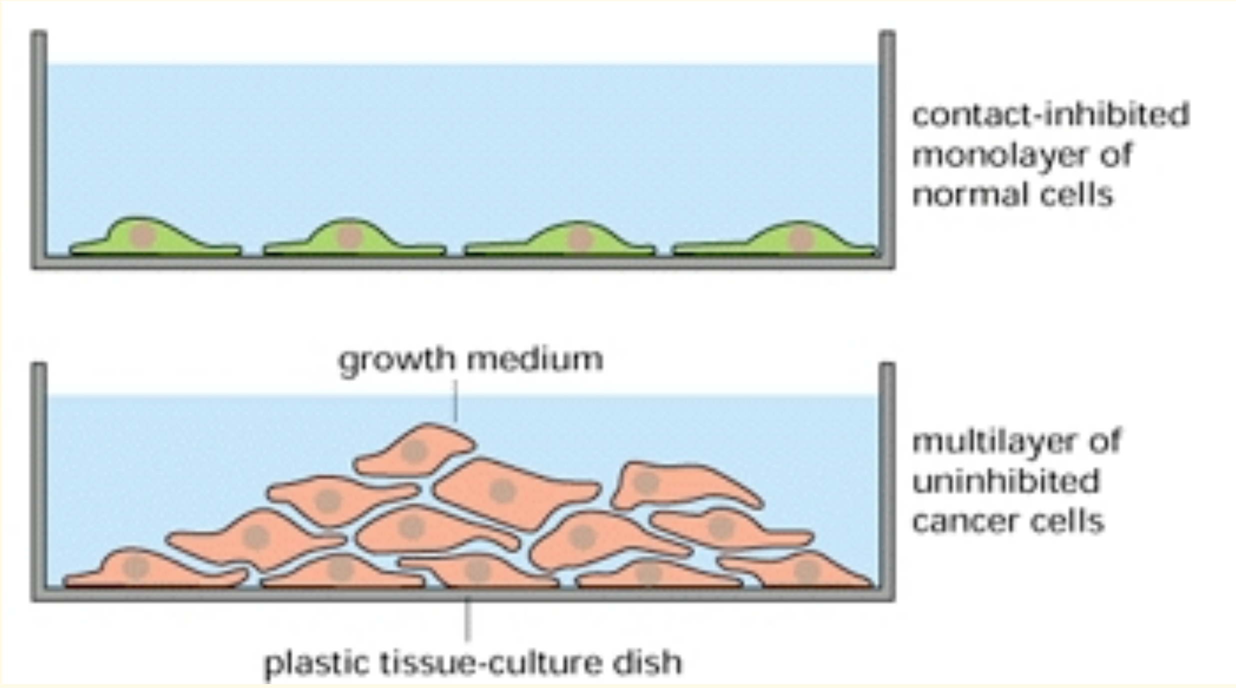
David Rogers,Vanderbilt University.



Jonathan Ashmore, University College London.



Cell 126, 677 (2006)

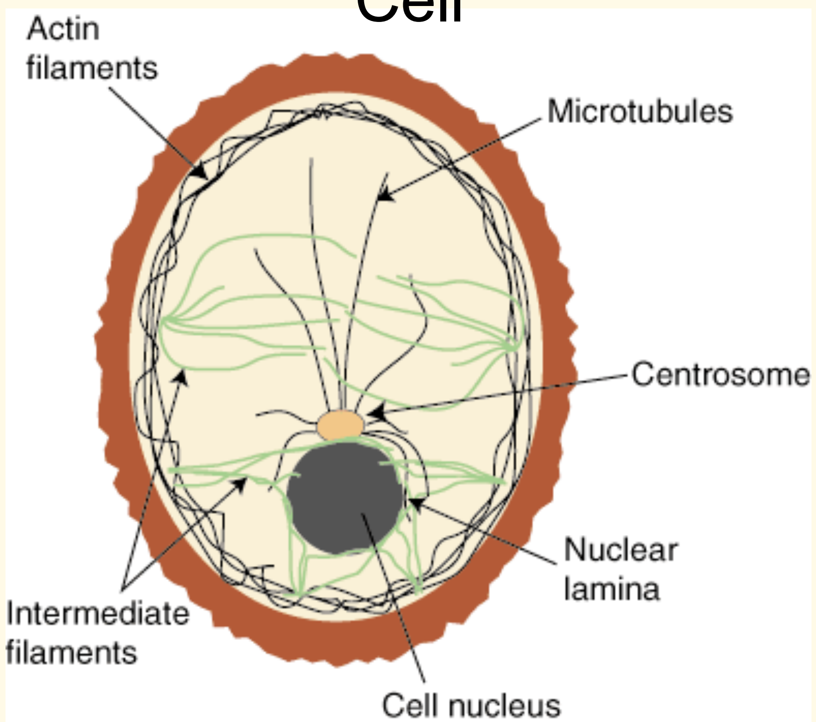


Nature Reviews Molecular Cell Biology 9, 603 (2008)

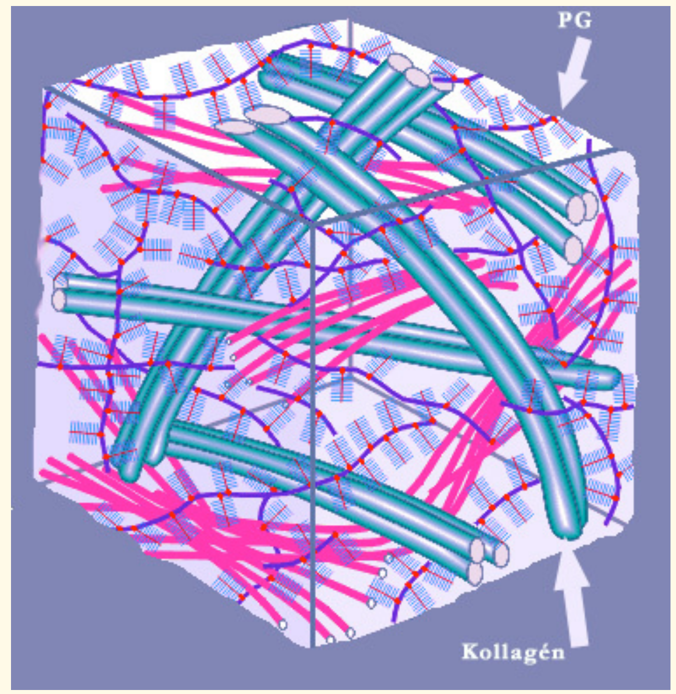
Cell mechanics

Cell cytoskeleton, its interaction with extracellular matrix (ECM)

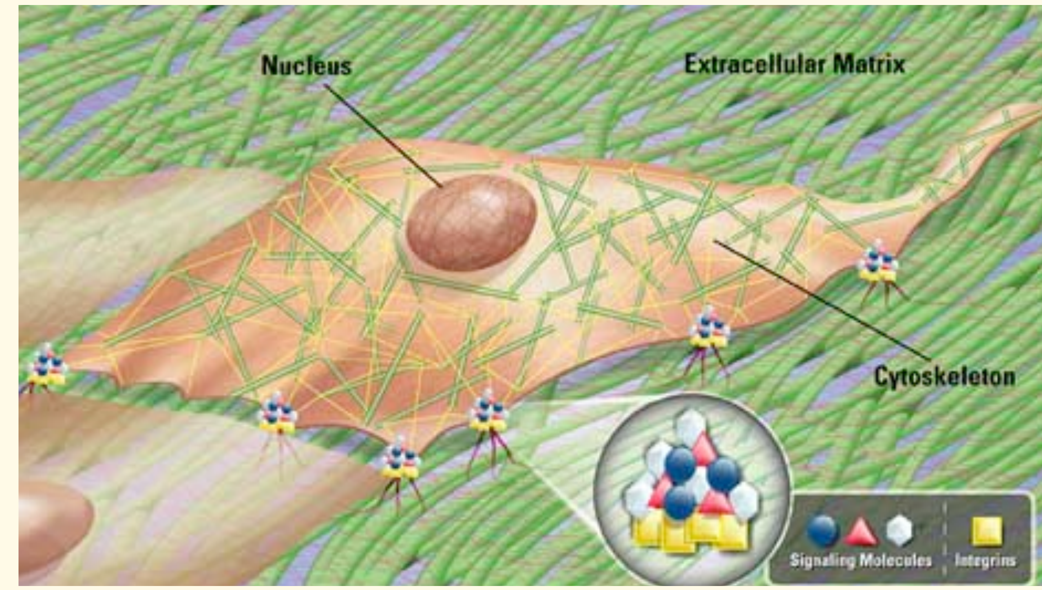
Cell



ECM



Cell + ECM



Cytoskeleton as a composite:

Synergistic mechanical coupling between different types of biopolymers

M.D., A.J. Levine and F.C. MacKintosh, **Europhys. Lett.** 84, 18003 (2008).
M.D. and F.C. MacKintosh, **Phys. Rev. Lett.** 105, 138102 (2010).
M.D. and F.C. MacKintosh, **Phys. Rev. E.** 84, 061906 (2011).

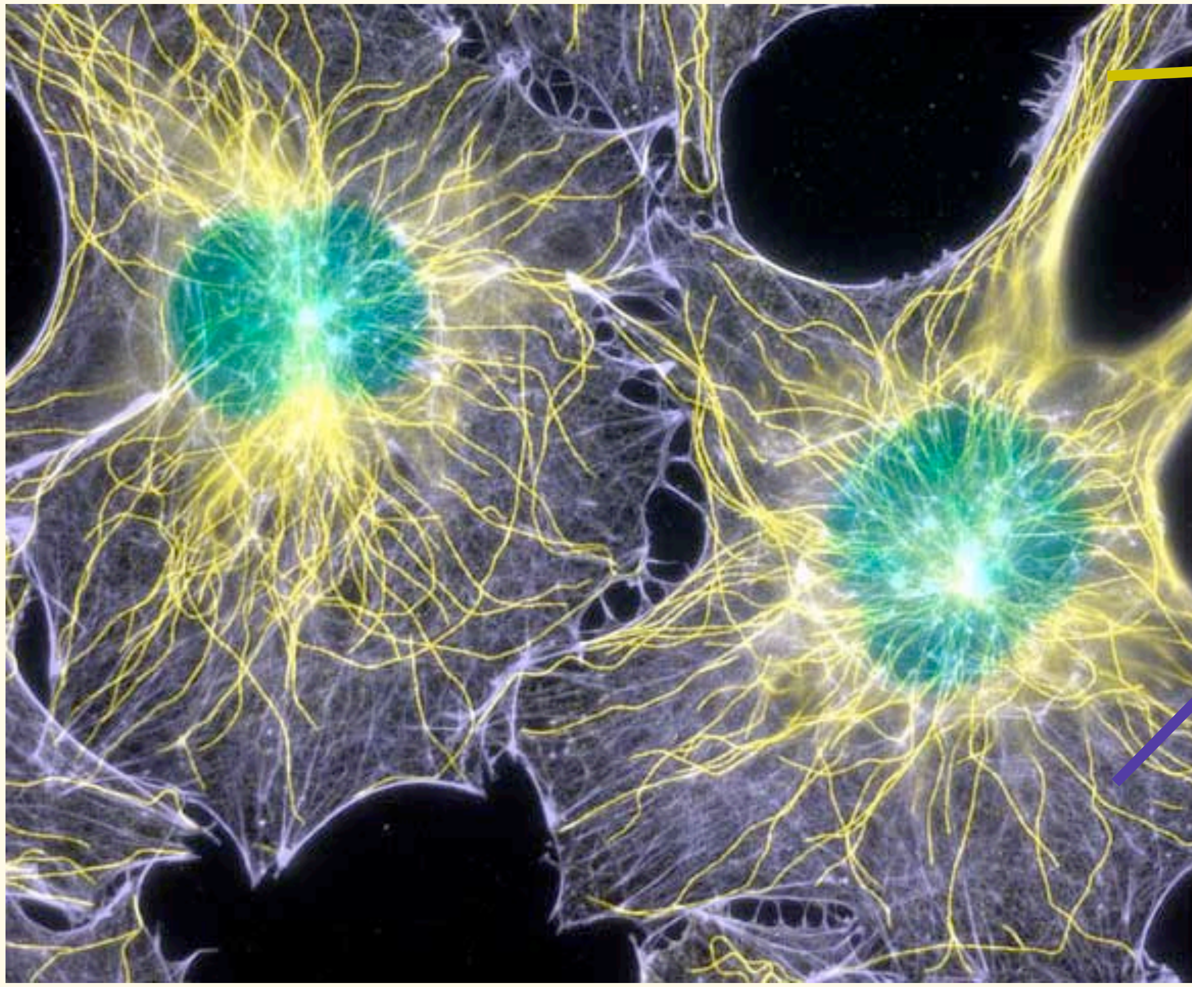
Cooperative interaction between different types of crosslinkers

M.D., D. Quint and J.M. Schwarz, **PLoS ONE** 7, 35939 (2012).

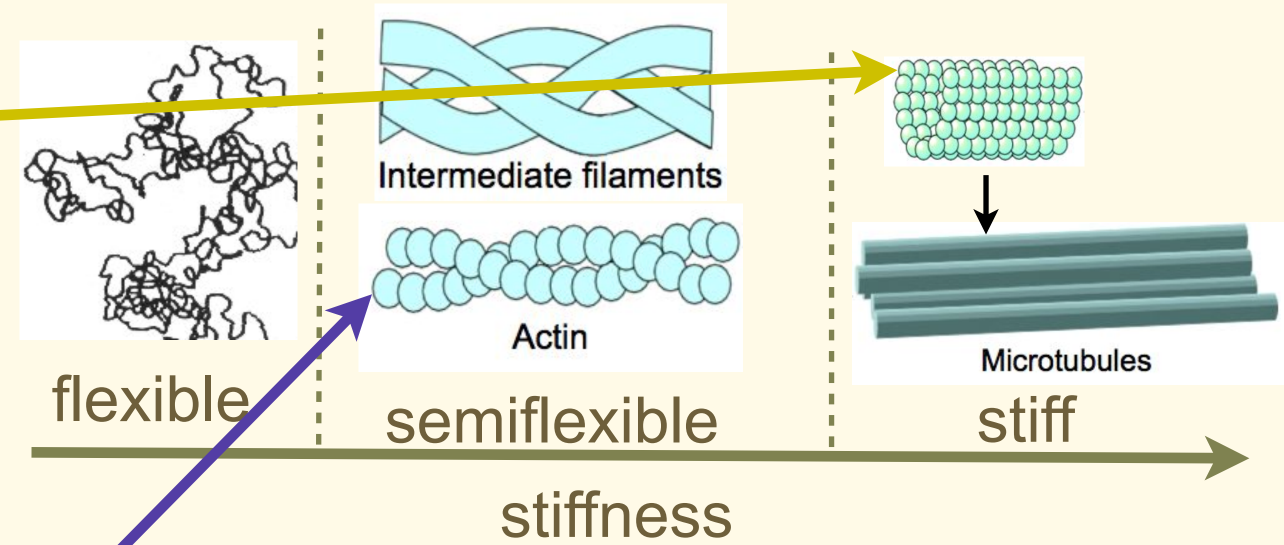
The extracellular matrix as a composite: structure function relations

J.L.Silverberg, **M.D.**, L. Bonassar, and I. Cohen, *in preparation*

Cytoskeleton as a composite



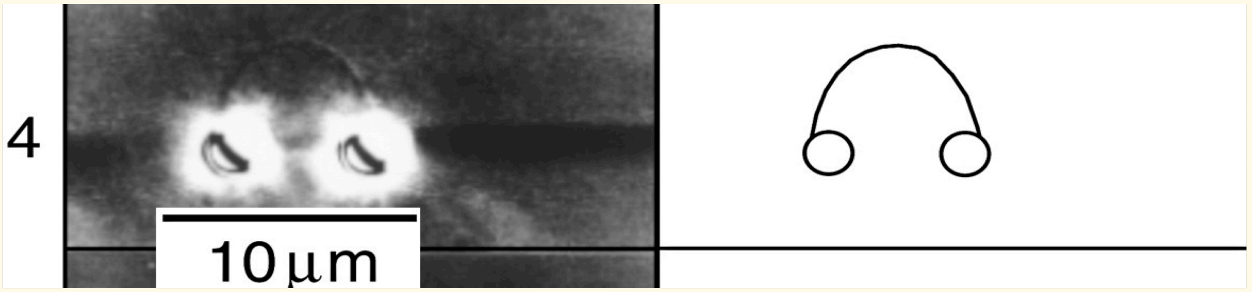
From Cell: An Image Library (Image by Torsten Wittmann).



flexible

- Three major types of filaments: **microtubules (MTs)**, **filamentous actin (F-actin)** that forms a 3d network, intermediate filaments.
- F-actin and MTs have very different mechanics.
- F-actin crosslinked with multiple types of crosslinkers: stiff, flexible & freely rotating, flexible & angle constraining.

How large of forces can microtubules carry? How far?

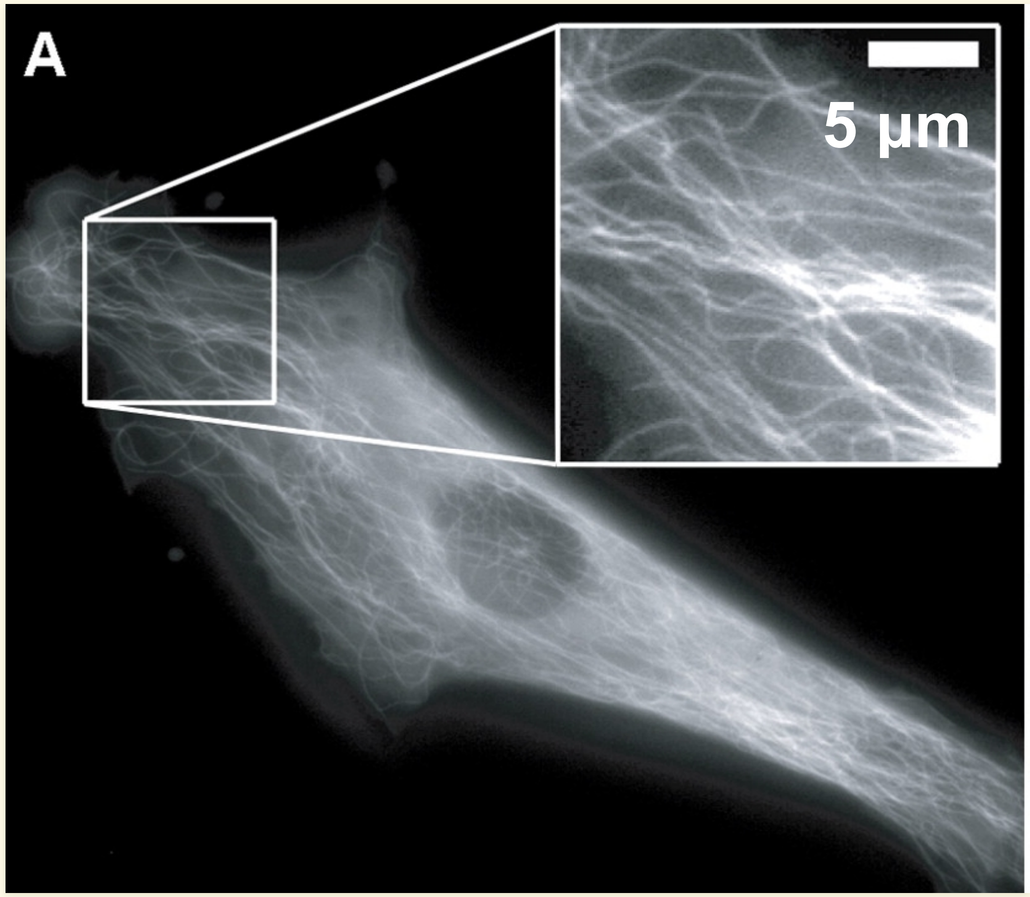


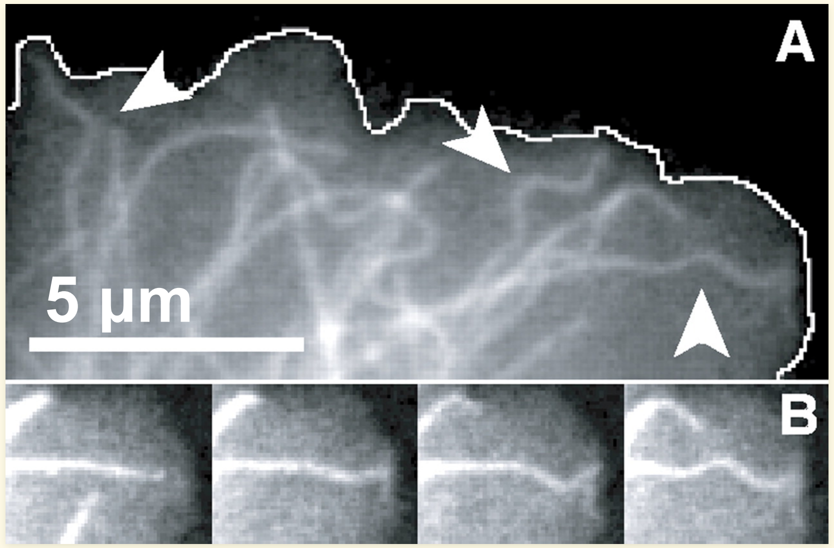
Kikumoto *et al.*, Biophys J, 2005

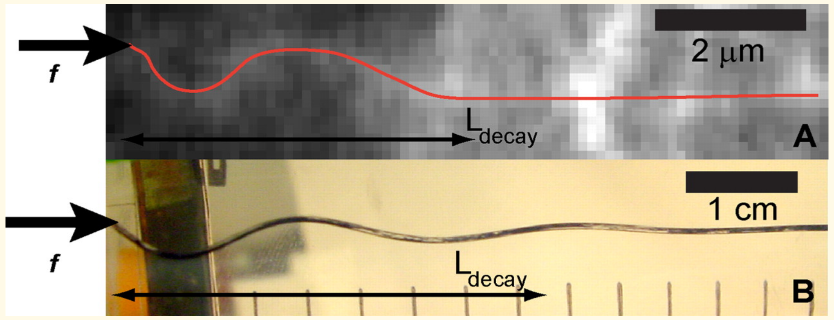
Isolated, in vitro:

Buckling at large wavelength ~ 10 µm.
Can be explained by classical Euler buckling.

In Cells







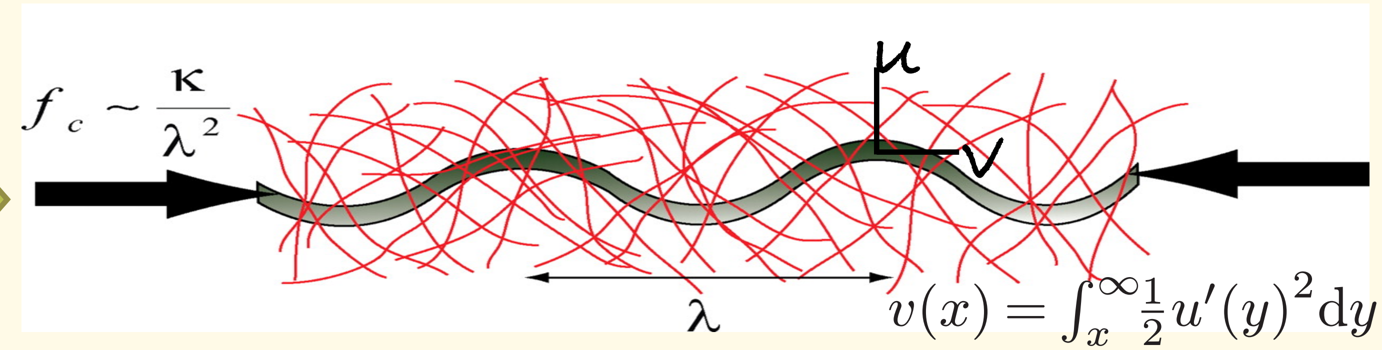
Brangwynne *et al.* J. Cell. Bio. **173**, 733 (2006).

- Resist compressive forces at least 100 times more than in vitro.
- Buckle at much shorter wavelengths ~ µm, show attenuation

Mechanics of Microtubules in cells : Nonlinear Elastic Theory

M.D., A.J. Levine and F.C. MacKintosh, **Europhys. Lett.** 84, 18003 (2008).

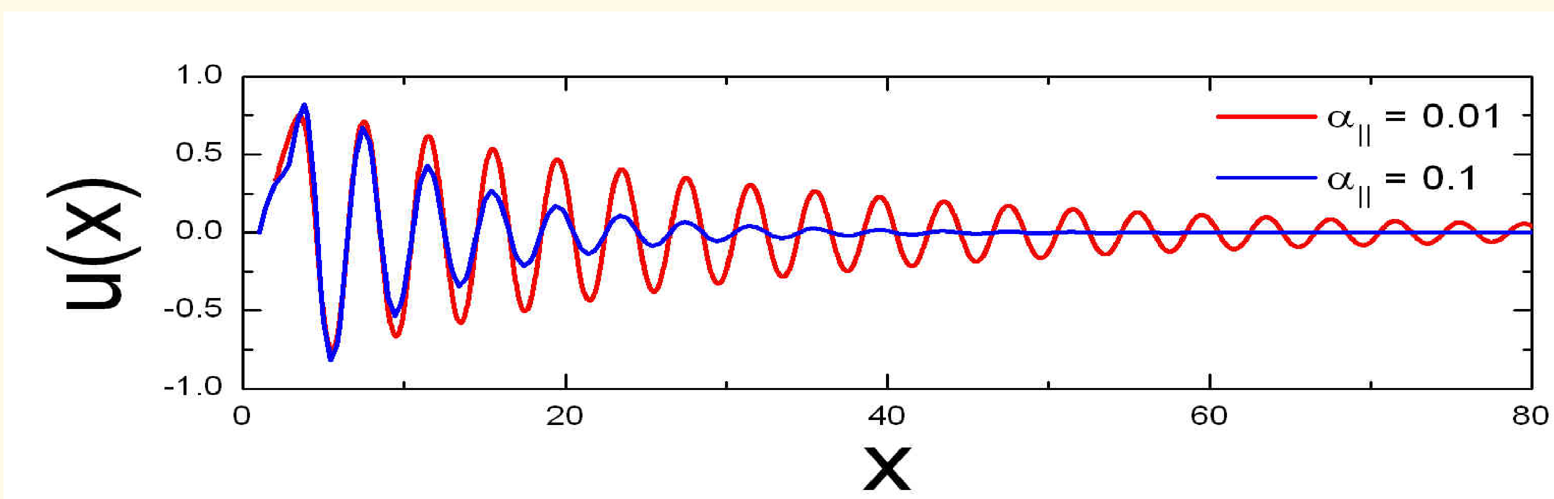
+

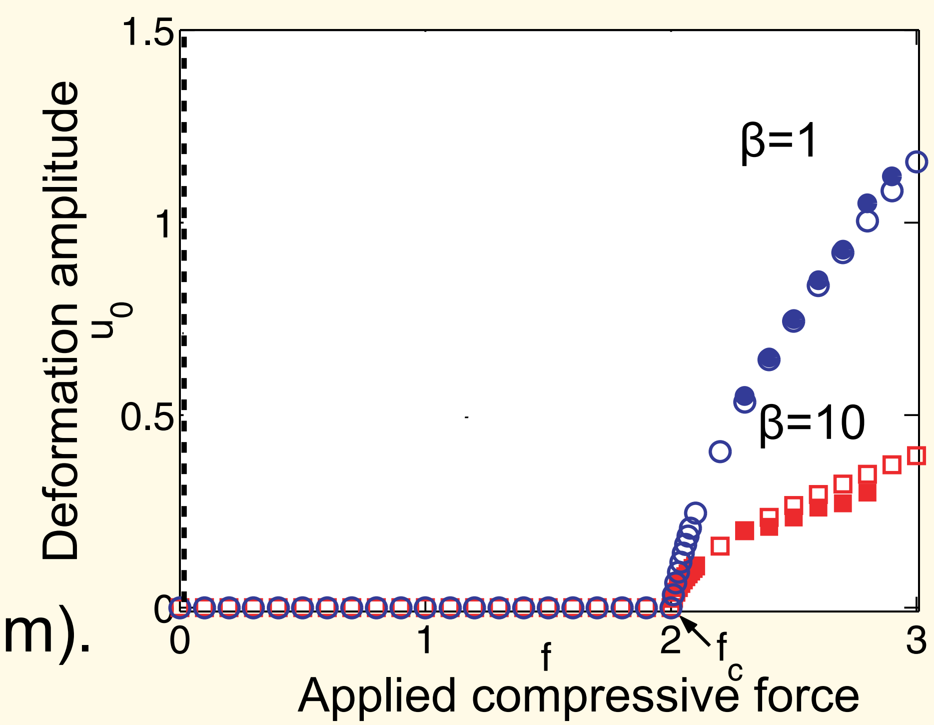


Deformation Energy of MT: Axial compression + Bending + coupling with F-actin matrix

$$\mathcal{E} = -fv(0) + \int_0^\infty \left[\frac{\kappa}{2} u''(x)^2 + \frac{\alpha_\perp}{2} u(x)^2\right] dx + \int_0^\infty \left[\frac{\alpha_\parallel}{2} v(x)^2 + \frac{\beta}{4} u(x)^4\right] dx$$

Use Variational calculation, Energy Minimization (simulation), Scaling Analysis

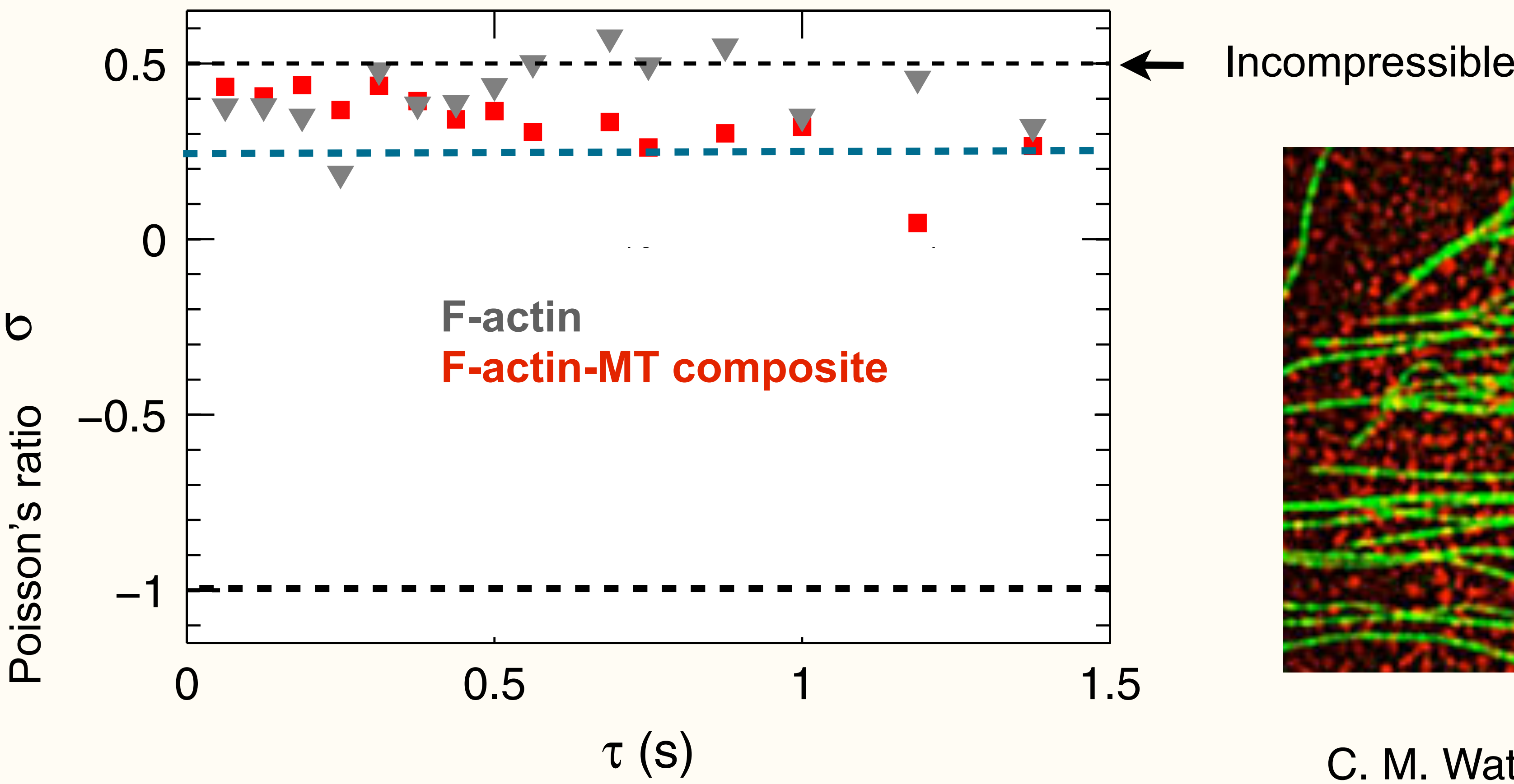




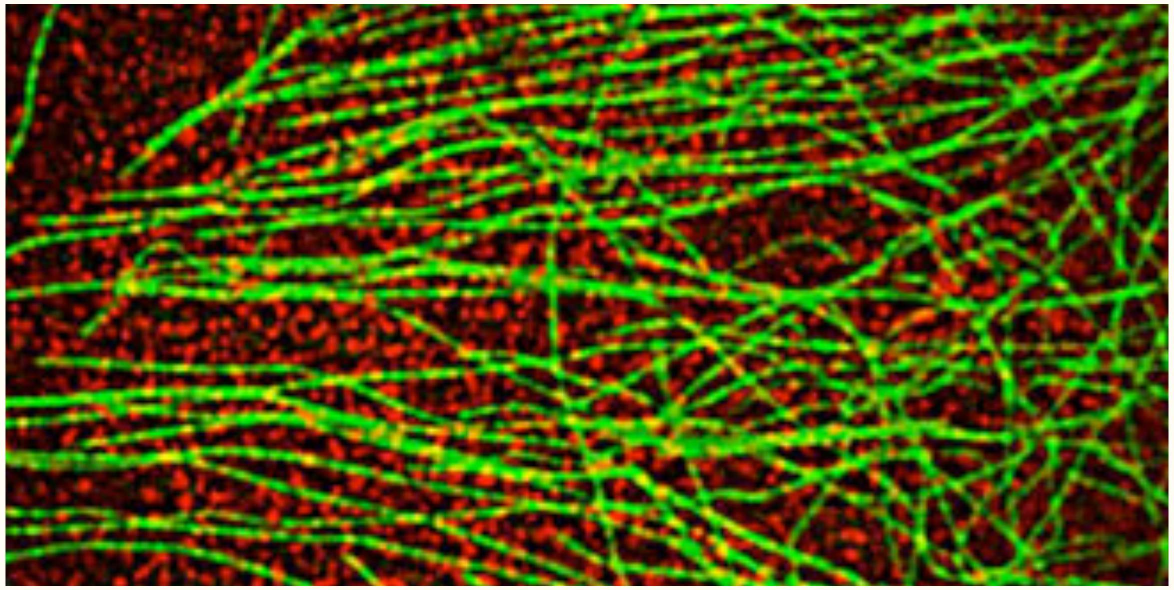
- Intracellular MTs show short wavelength buckling (λ~2μm).
- Can bear large compressive loads ~ hundreds of pN.
- Range of transmission ~ tens of microns, controlled by coupling to actin matrix.

Micromechanics of composites of F-actin and Microtubules (MTs)

- MTs significantly enhance stiffness of composite. [Lin *et. al.* , Soft Matter **7,** 902 (2011)]
- 2 pt microrheology of composite of F-actin (incompressible) + MTs : Poisson's ratio < 0.5 ⟶ compressible [Pelletier et. al. Phys Rev Lett. **102**, 188303 (2009)]



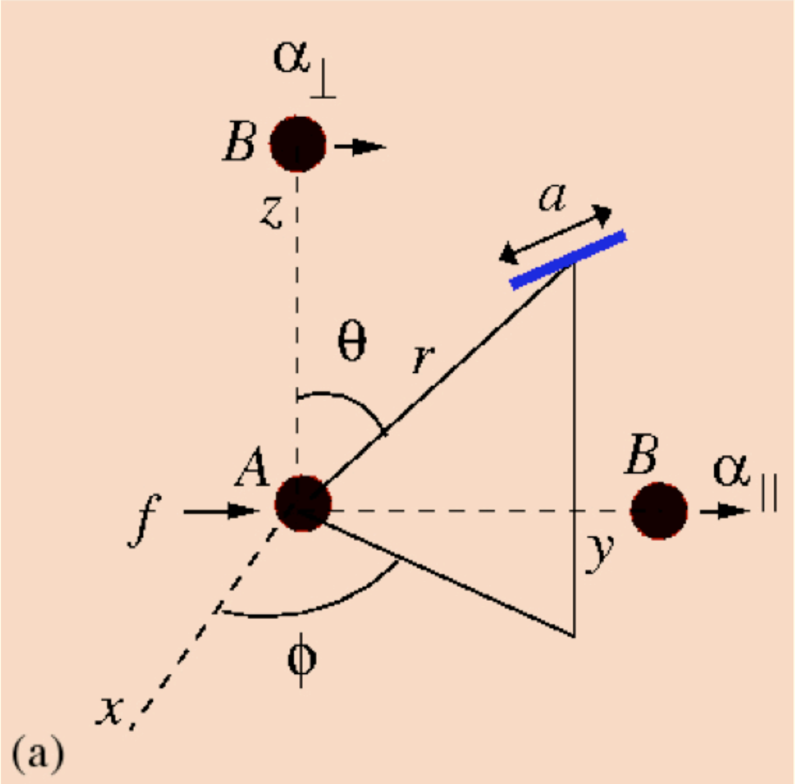
Pelletier et. al. Phys Rev Lett. **102**, 188303 (2009)



C. M. Waterman, Cytomechanical Module Abstracts, 2003.

Can not be understood by studying one-component filament networks

Effective Medium Theory



F-actin: Elastic background + MTs: rods

- Measure of compressibility, $\boldsymbol{\beta = \mu / (\lambda+2\mu)}$
- Linear response theory:

Displacement field at r due to force at r' :

$$u_i(\vec{r}) = \alpha_{ij}(\vec{r}-\vec{r}')\,F_j(\vec{r}') \quad \text{where} \quad \alpha(\vec{r}-\vec{r}') = \alpha_{||}(\vec{r}-\vec{r}')\,r_i r_j + \alpha_\perp(\vec{r}-\vec{r}')\,(1-r_i r_j)$$

$$\alpha_{||}=1/4\pi\,|\vec{r}-\vec{r}'|G, \quad \alpha_\perp=(1+\beta)\,\alpha_{||}/2.$$

- Dipole approximation for rods.
- Evaluate dipole field at location of B, consequent change in linear response.

$\delta\lambda = \delta\mu = (\pi/30)\mu a^3\,\delta n$

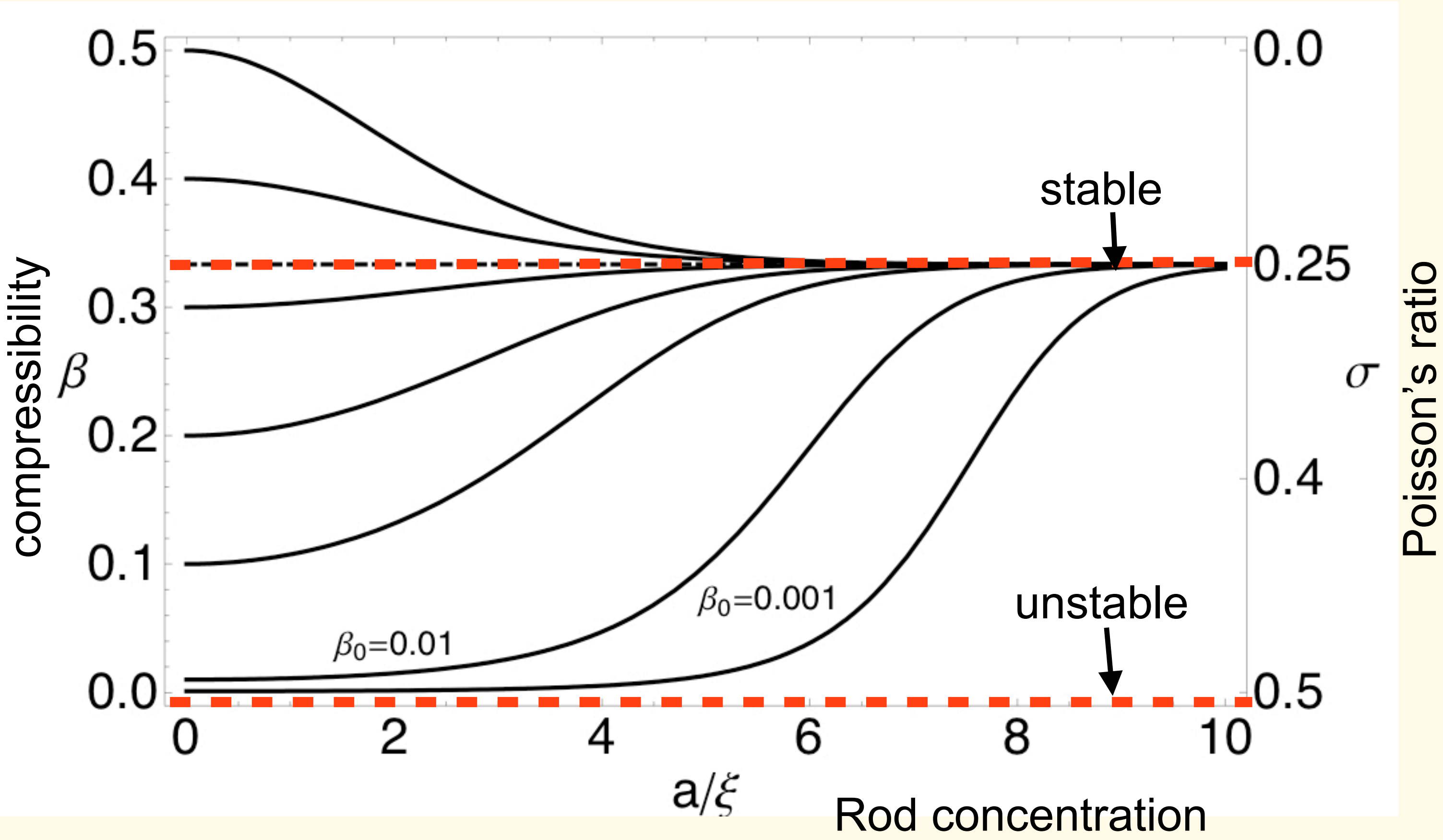
$\delta\lambda = \delta\mu = (\pi/30)\mu a^3\,\delta n/(1 + \mu/\mu_{rod})$, for finite rod compliance.

$\delta\beta = (\pi/30)\,a^3\,\beta(1-3\beta)\,\delta n$

Same results can also be obtained using a more continuum approach.

M.D. and F.C. MacKintosh, **Phys. Rev. Lett.** 105, 138102 (2010).

Poisson's ratio, compressibility approach stable fixed point with increase in rod density



M.D. and F.C. MacKintosh,
Phys. Rev. Lett. 105, 138102 (2010)

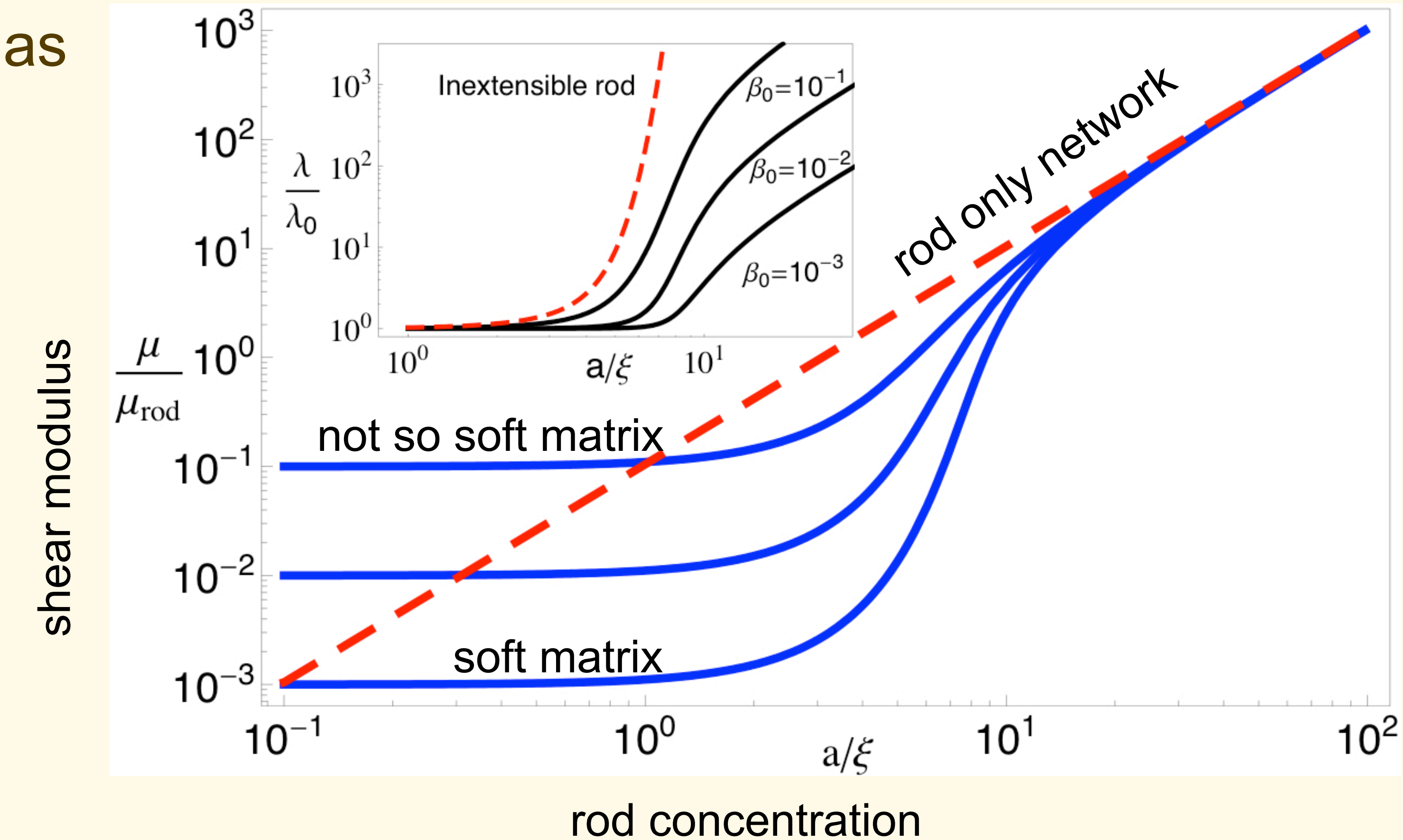
M.D. and F.C. MacKintosh,
Phys. Rev. E. 84, 061906 (2011).

Shear modulus increases with rod density as

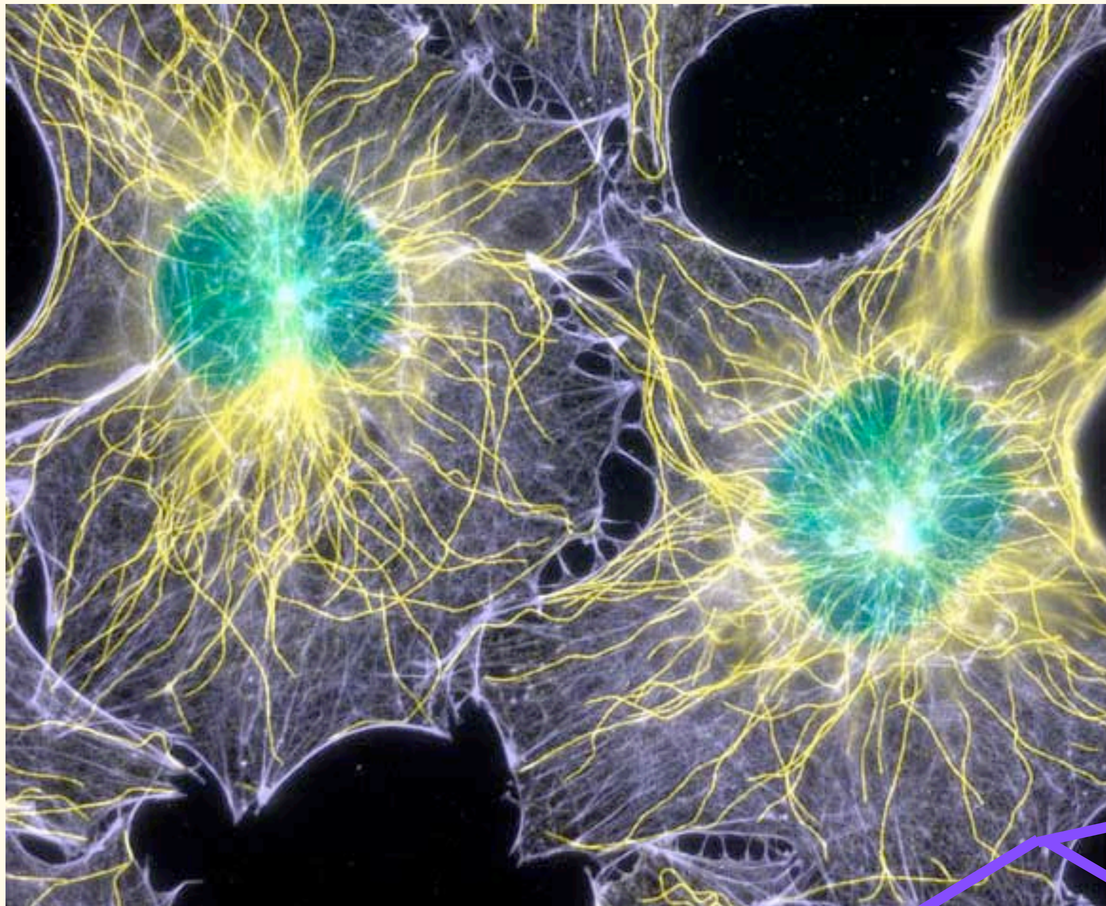
For inextensible rods,
$\mu = \mu_0 \exp(\pi n a^3/30)$

For extensible rods,
$\mu = \mu_{rod} W[\mu_0/\mu_{rod} \exp(\pi n a^3/30) \exp(\mu_0/\mu_{rod})]$

Affine result at high rod densities.



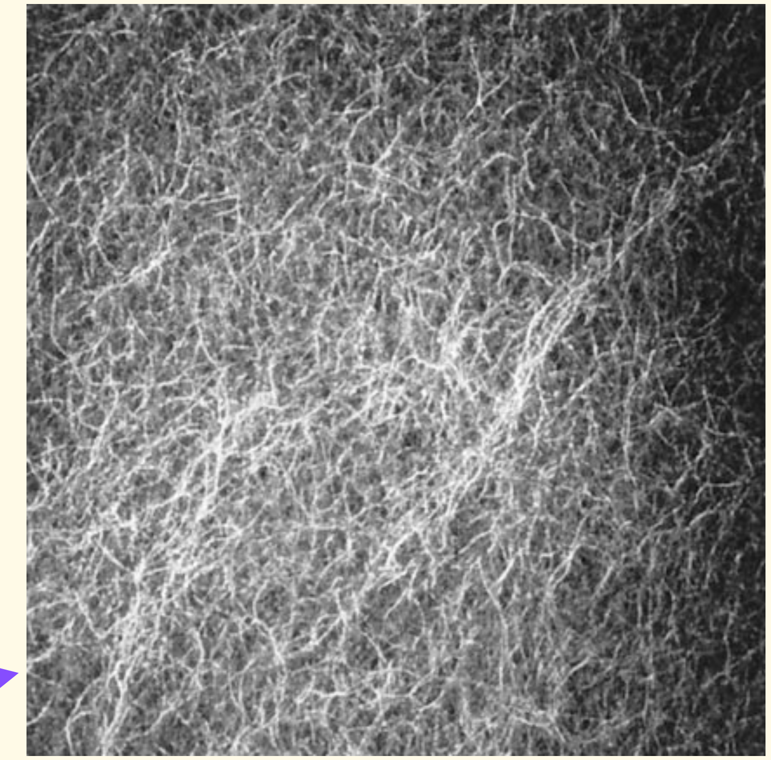
Actin cytoskeleton: many types of crosslinkers



from: Cell Image Library (by Torsten Wittmann).

Freely rotating crosslinkers

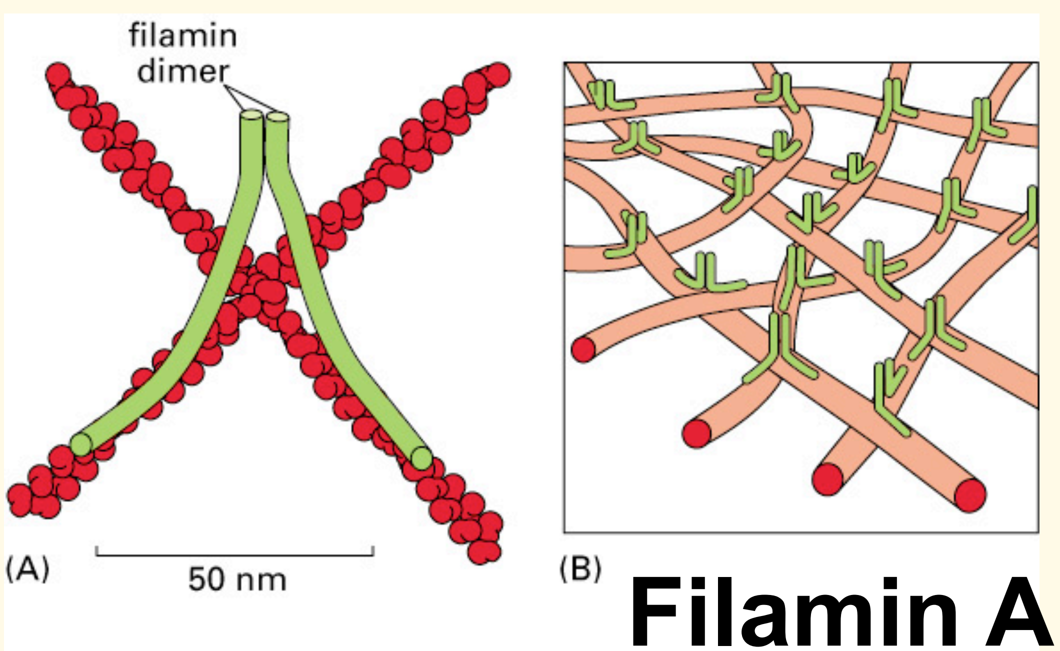
α- actinin



F-actin/a-actinin network: confocal micrograph Lower side: 120 μm. Tseng, Wirtz 2001.

See also DS Courson et al, Biol. Chem (2010).

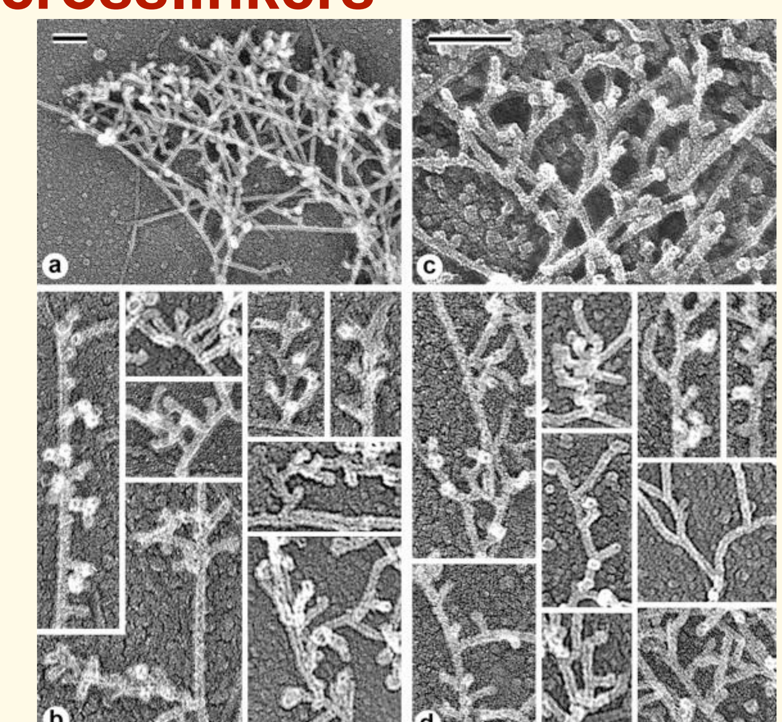
Angle constraining crosslinkers



Filamin A

Figure 16–42. Molecular Biology of the Cell, 4th Edition.

See also F Nakamura et al., J. Cell Biol (2007).



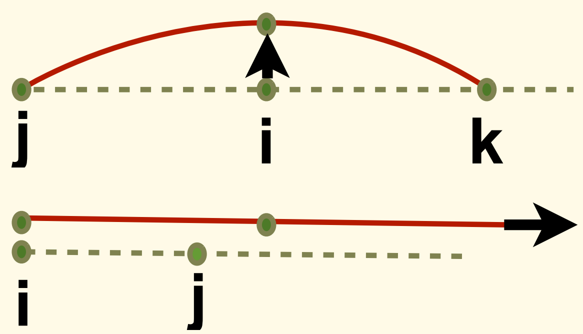
Arp 2/3

EM of keratocyte, fibroblast lamellipodial actin network, Svitkina and Borisy 1999.

Effective medium theory of bond diluted networks

M.D., F.C. MacKintosh and A.J. Levine, Phys. Rev. Lett. **99**, 038101(2007).
M.D., D. Quint and J.M. Schwarz, PLoS ONE **7**, 35939 (2012).

- Arrange infinitely long filaments in plane of 2D lattice.
- Each filament: extensional spring constant and bending rigidity.



Single filament properties :
Assume linear elasticity, $|u|/|R| << 1$

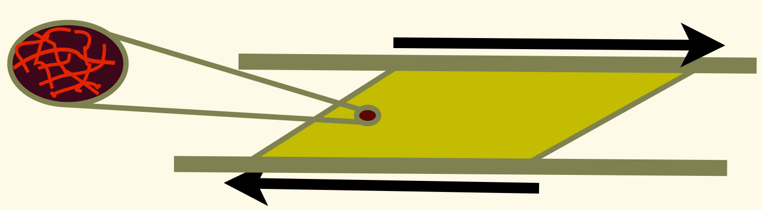
Bending $\quad 1/2 \ \kappa R^{-2} \sum (\mathbf{u}_{ij} \times \hat{r}_{ij} - \mathbf{u}_{ik} \times \hat{r}_{ik})^2$

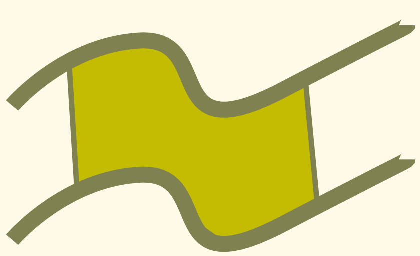
Stretching $\quad 1/2 \ \alpha \sum (\mathbf{u}_{ij} \cdot \hat{r}_{ij})^2$

- Finite filament length L introduced by cutting bonds with probability 1- p ($0<p<1$).
  No correlation between cutting points.
- Two types of cross-links

(a) Cross-links do not constrain angle between crossing filaments.
(b) Cross-links constrain angle with a finite stiffness.

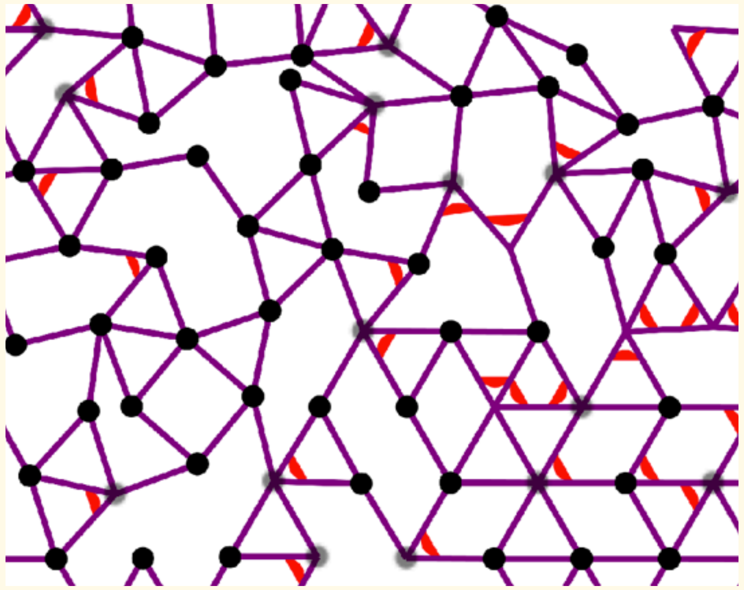
- Study mechanical response of this disordered network in linear response regime.

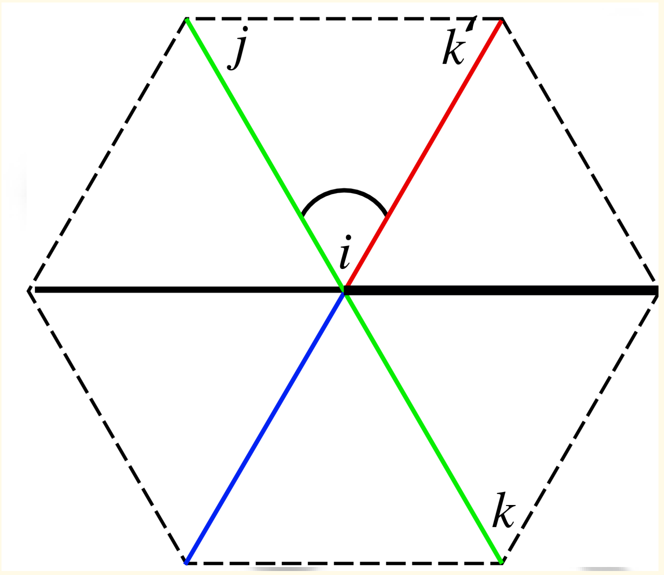




Effective medium elastic constants determined by requiring strain fluctuations produced in ordered network by cutting filaments have zero average.

Model: Semiflexible network with two crosslinker types



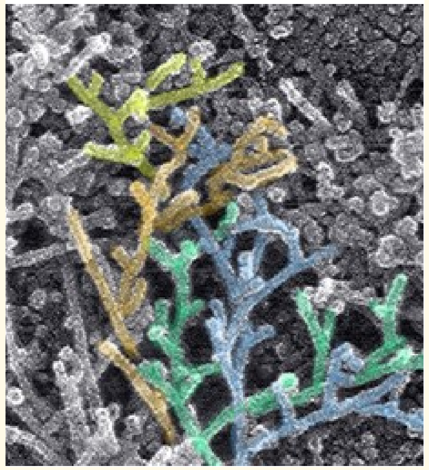


$$E = E_{spring} + E_{filament\ bending} + E_{non-collinear\ bending}$$

$$E_{spring} = \frac{\mu}{2} \sum_{\langle ij \rangle} p_{ij} (\vec{u}_{ij}.\hat{r}_{ij})^2$$

$$E_{filament\ bending} = \frac{\kappa}{2} \sum_{\langle jik \rangle} p_{ji} p_{ik} (\theta_{jik})^2$$

$$E_{non-collinear\ bending} = \frac{\kappa_{nc}}{2} \sum_{\langle jik' \rangle} p_{ji} p_{ik'} p_{nc} (\Delta\theta_{jik'})^2$$

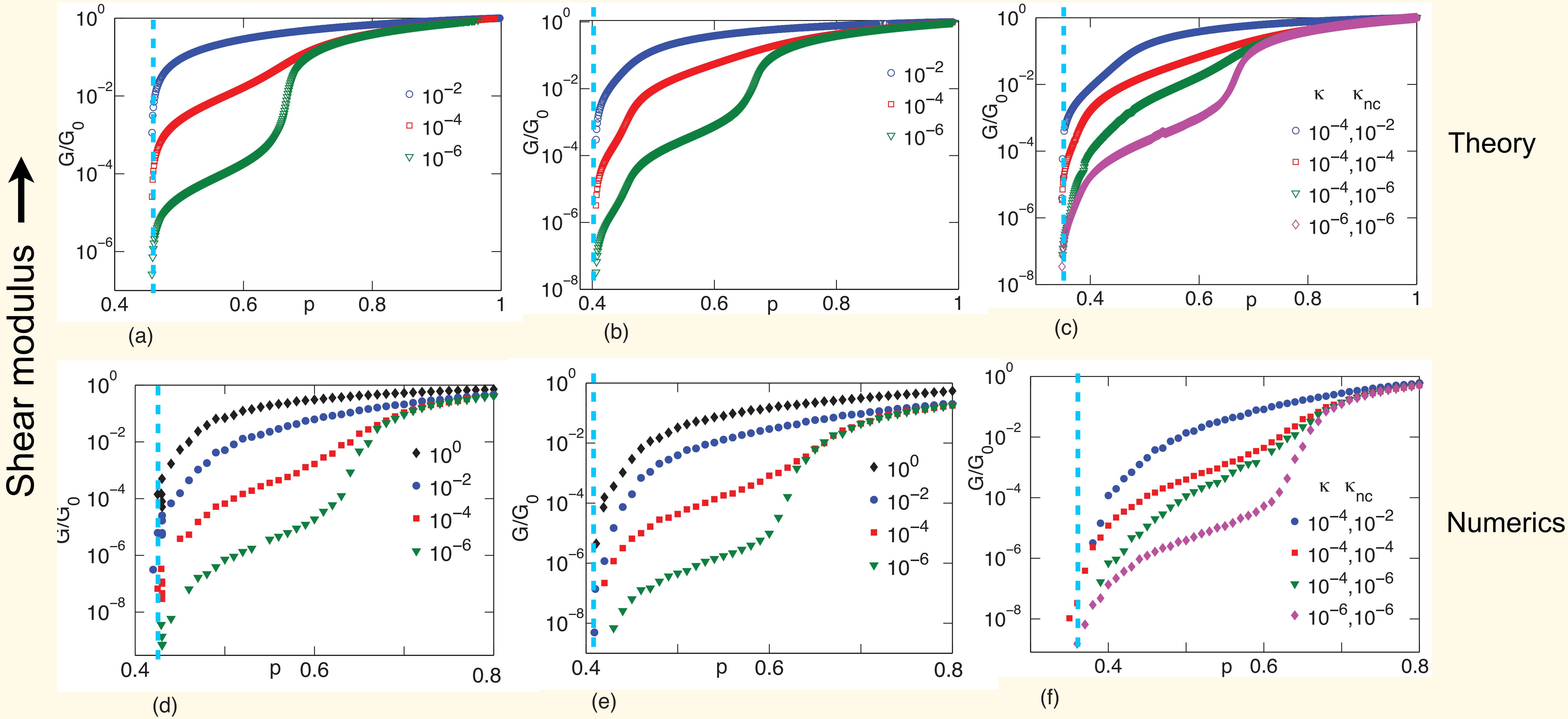


Study using Effective Medium Theory, Numerical Minimization.

M.D., D. Quint and J.M. Schwarz, PLoS ONE **7**, 35939 (2012).

Linear mechanical response

semiflexible network, free rotating x-links | spring network, multiple type xlinks | semiflexible network, multiple type xlinks



G_0 : shear modulus ordered network.

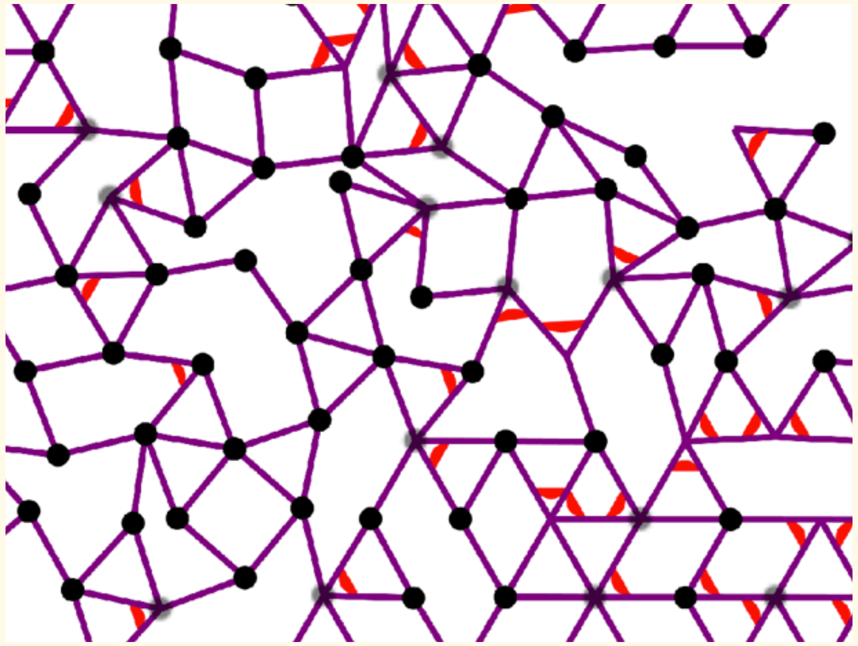
Bond occupation probability p →

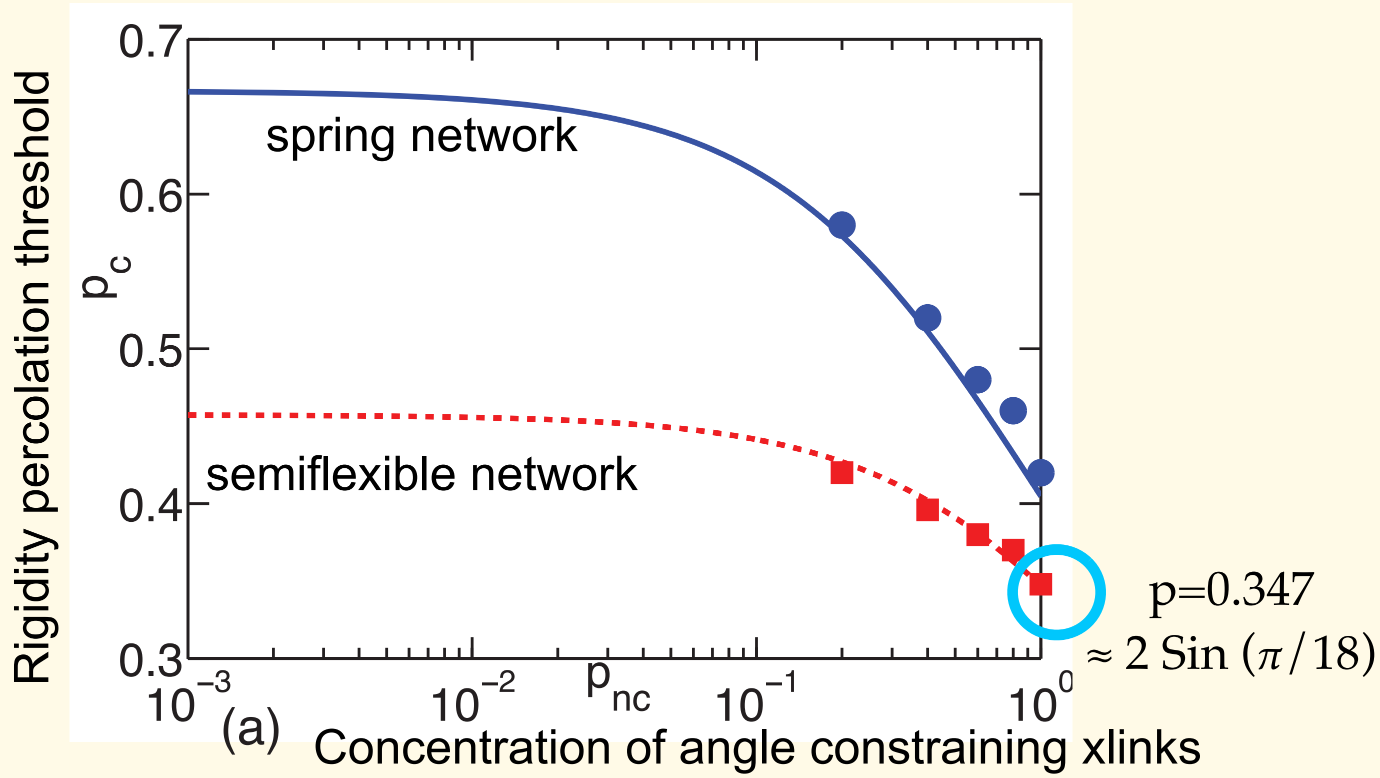
Average filament length = 1/(1-p)

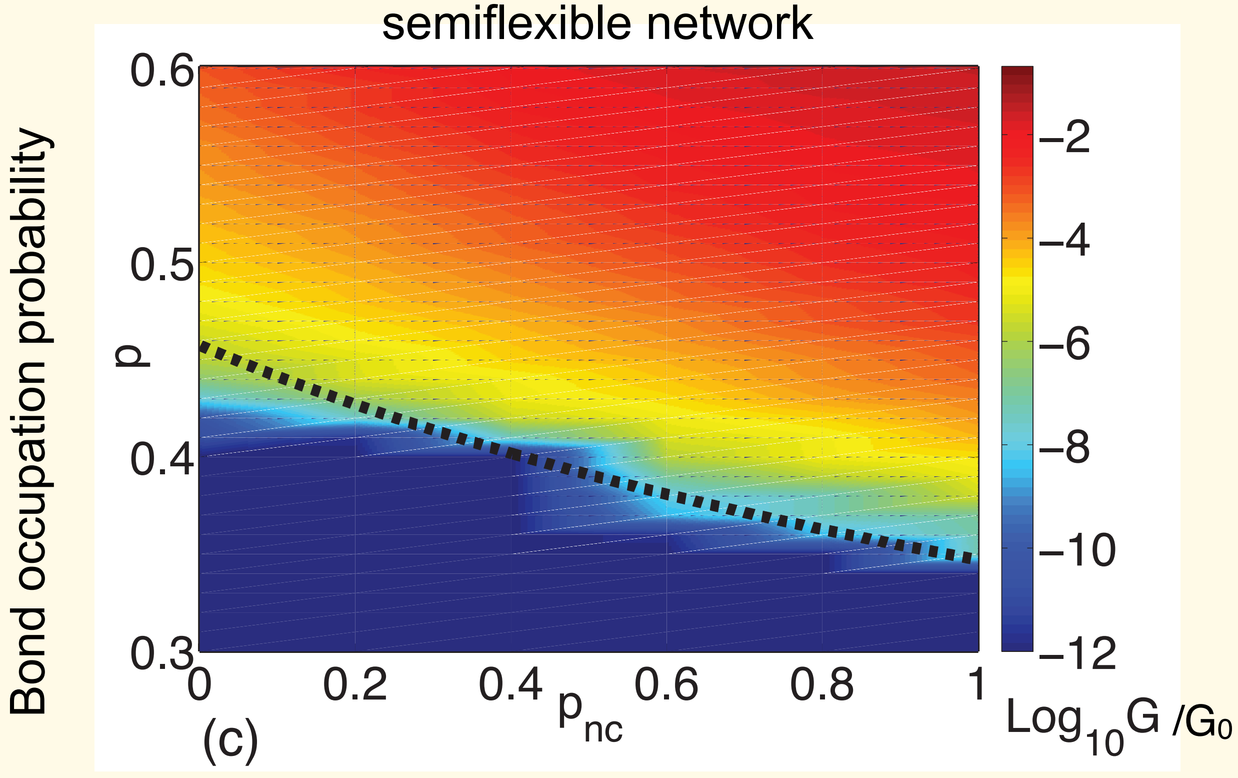
Qualitatively similar mechanics, different rigidity percolation thresholds

M.D., D. Quint and J.M. Schwarz, PLoS ONE **7**, 35939 (2012).

Angle constraining crosslinkers help attain rigidity with minimum amount of filamentous material







Concentration of angle constraining xlinks

Multiple crosslinkers cooperate to tune rigidity of cytoskeleton!

Cytoskeleton as a composite

Collective response of the cytoskeleton is highly synergistic

- F-actin enhances load bearing capacity of MTs, imparts them stability and allows stress propagation over large but finite distances.
- MTs impart the cytoskeleton enhanced compressibility relative to shear compliance, significantly increase the shear modulus of cytoskeleton making it more robust.
- Multiple crosslinkers in cytoskeletal networks show both redundant and cooperative mechanics. Can help tune cell mechanical response.

Important for cellular processes,

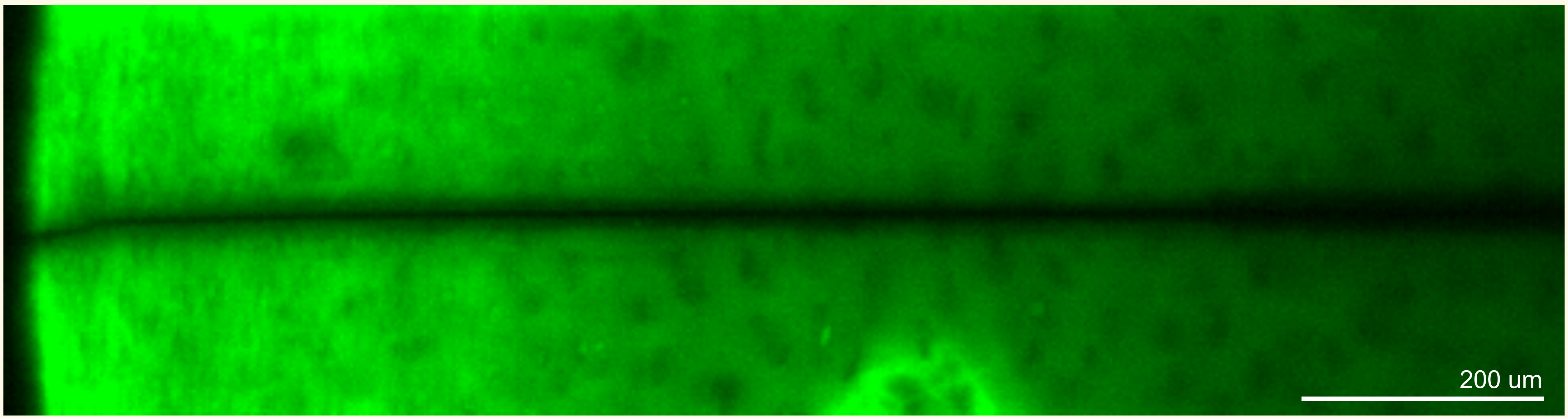
Novel and rich physics,

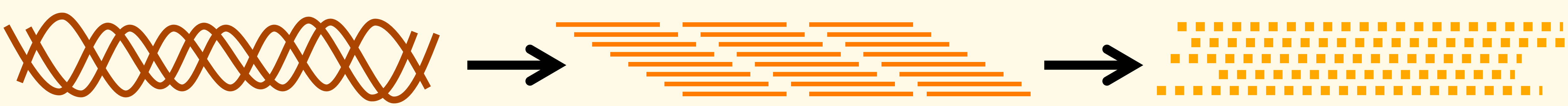
Wider elasticity context, applications for biomimetic materials.

Articular Cartilage Shear Mechanics

Structure, Function, and Variations

*Jesse L. Silverberg, **Moumita Das**, Lawrence Bonassar, Itai Cohen*



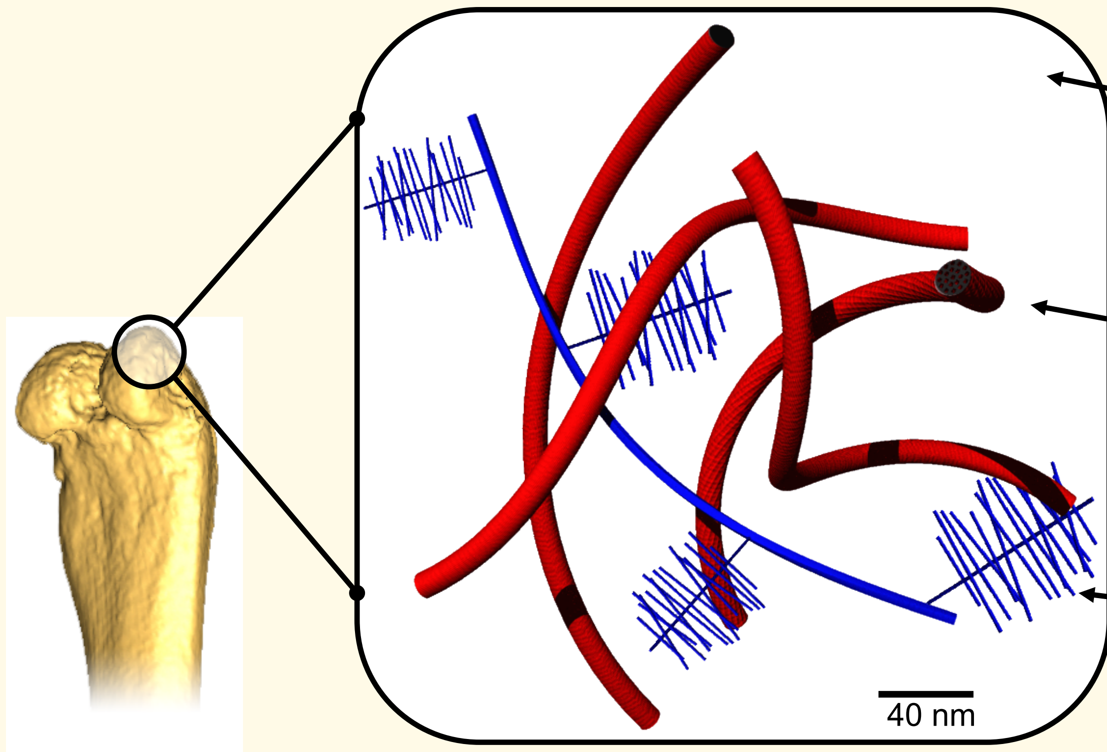


Collagen Molecules
L ~ 300 nm

Collagen Fibrils
L = 1 ~ 10 um

Collagen Fibers
L = 100 um ~ 1 mm

Articular Cartilage, in short



Water
60-85% wet weight

Collagen Fiber Network
endows tensile strength
15-22% wet weight

Aggrecan (PG) Network
osmotically swells tissue
4-7% wet weight

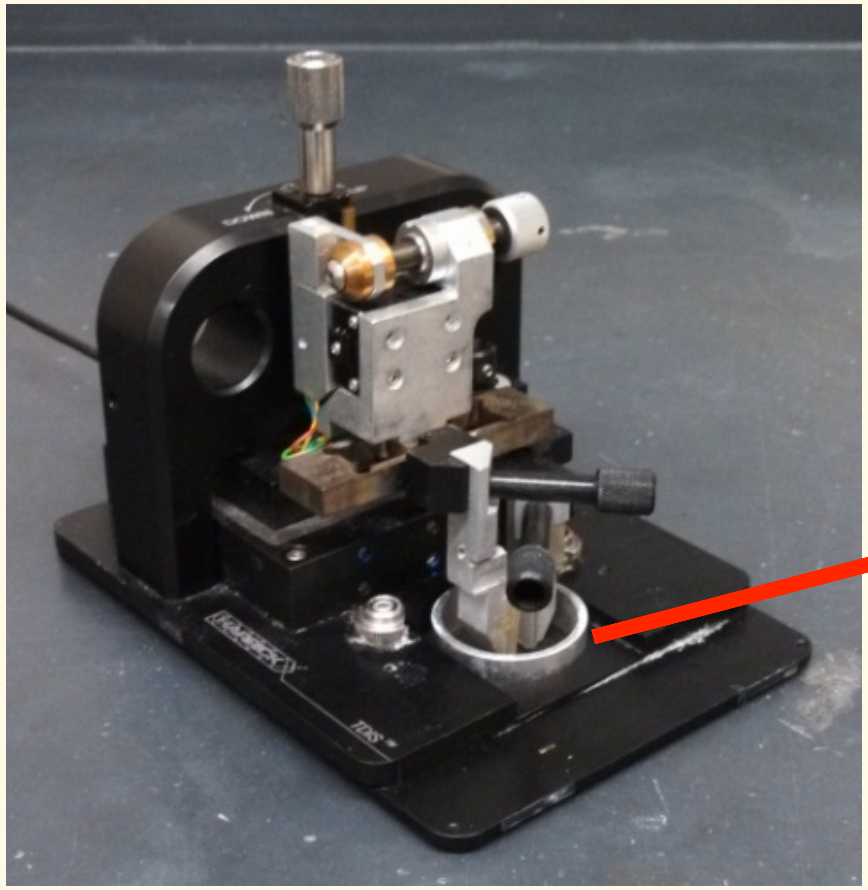
Quick Cartilage Facts:

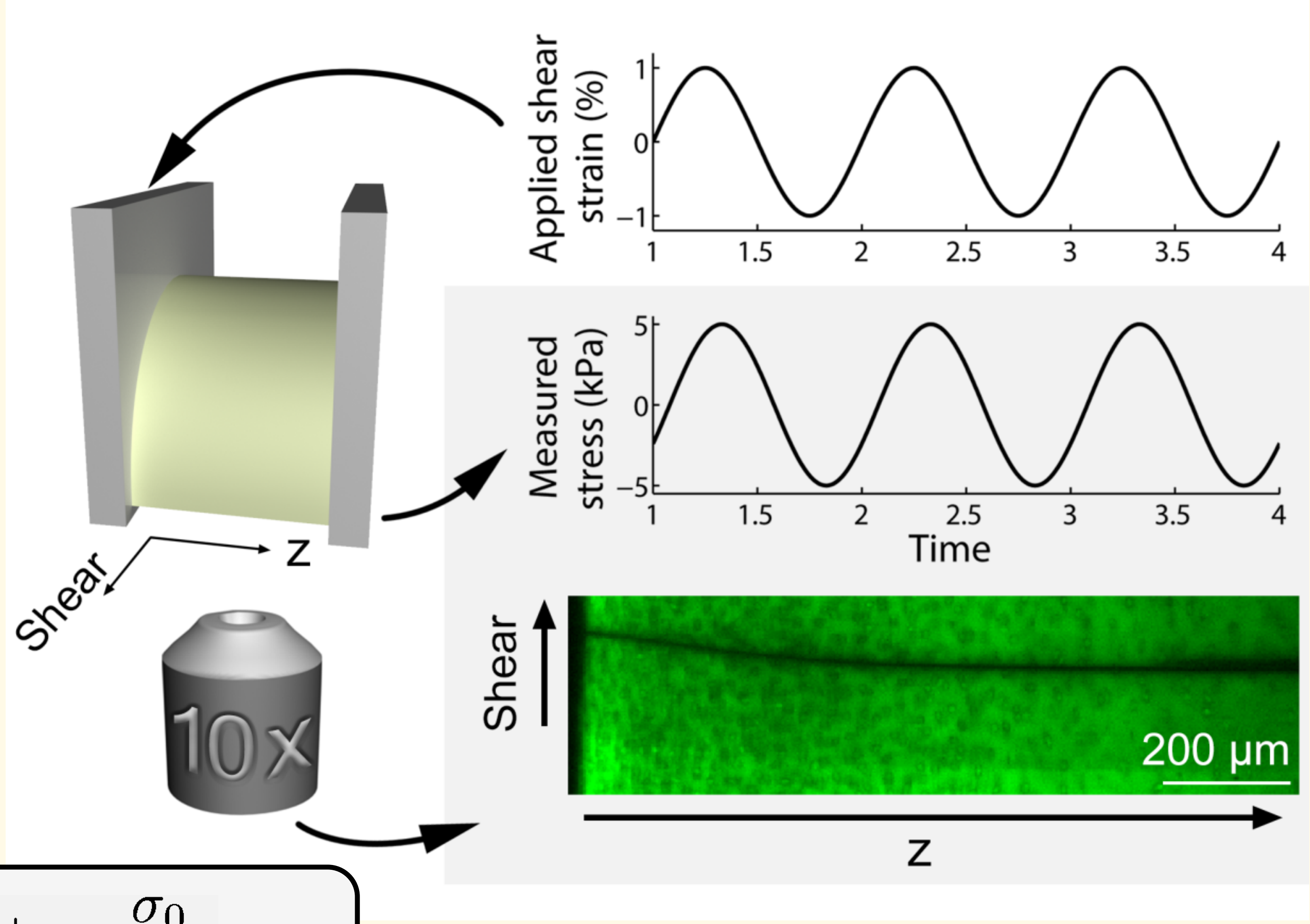
Multiscale self-assembled material:

Tropocollagen ~300 nm; Fibrils ~1 μm; Fibers ~10 μm
Loads exceed 10x body weight;
Life time: 7~8 decades; minimal self-repair

What enables cartilage to endure such extreme demands?

Confocal rheology maps local shear strain

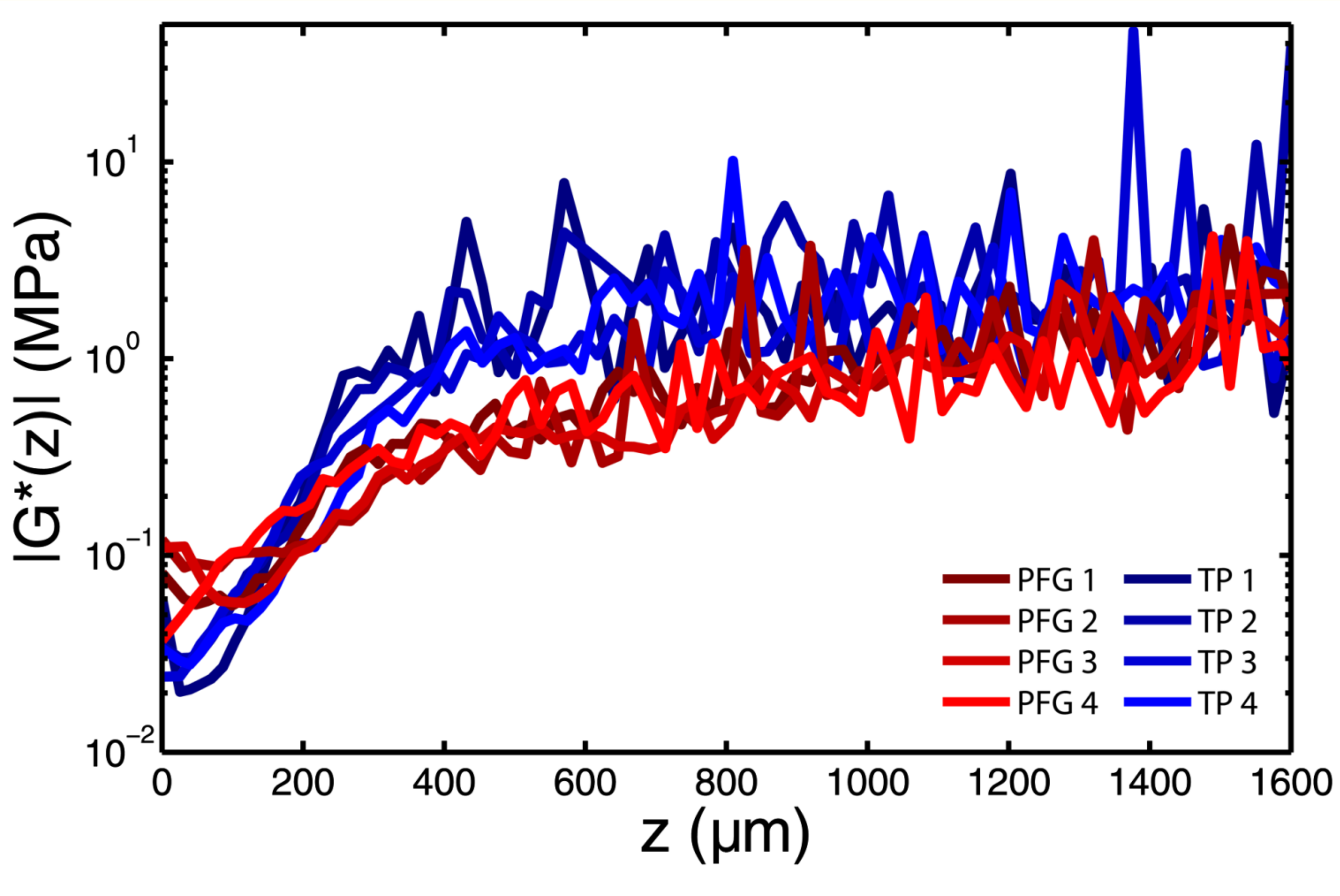




$$|G^*(z)| = \frac{\sigma_0}{\gamma(z)}$$

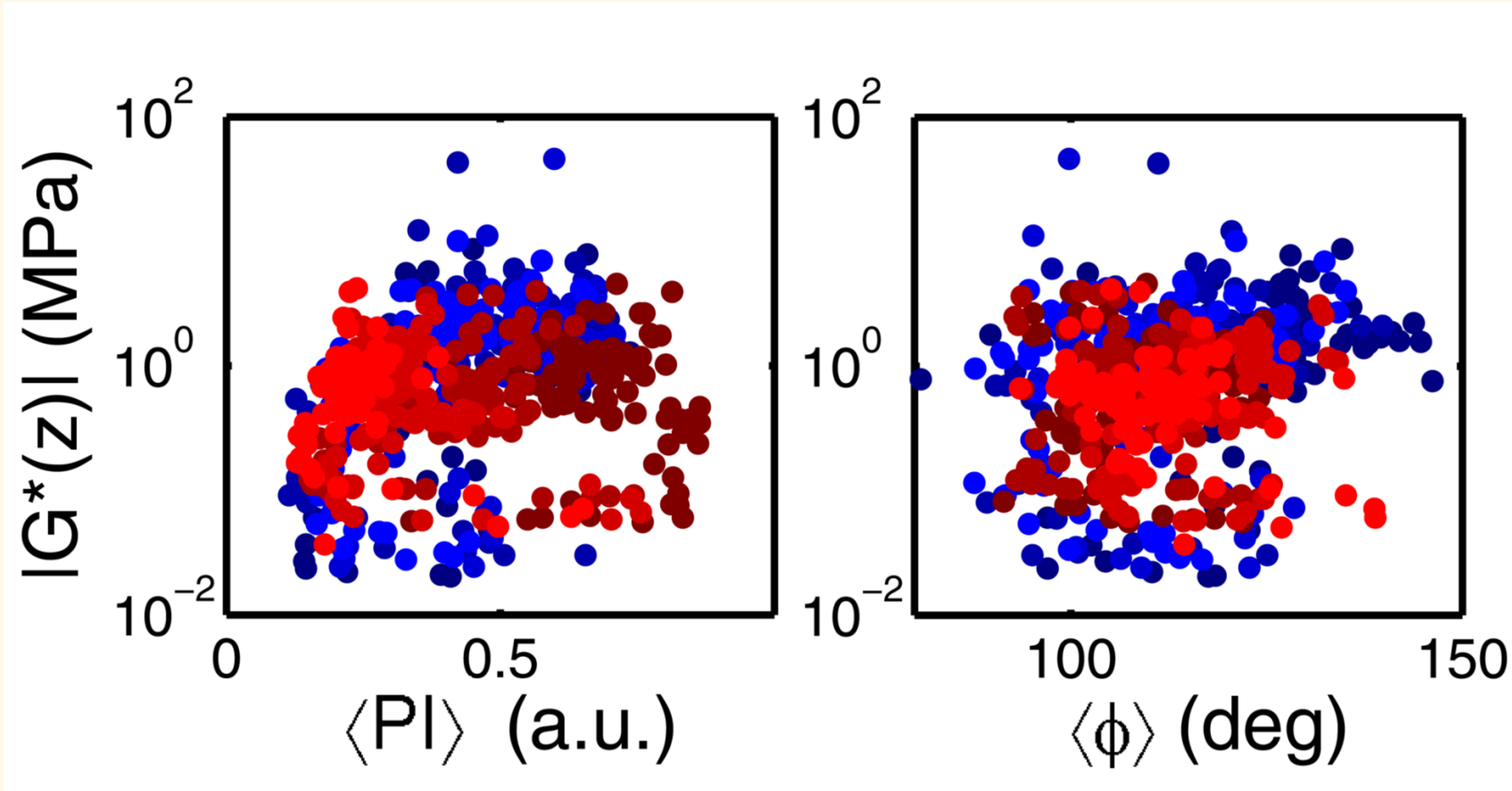
M Buckley et. al. *J Biomech* (2008, 2010), *J Biomech Eng* (2013)

Spatially resolved shear modulus |G*(z)| parallels collagen fiber organization ? (seems so)



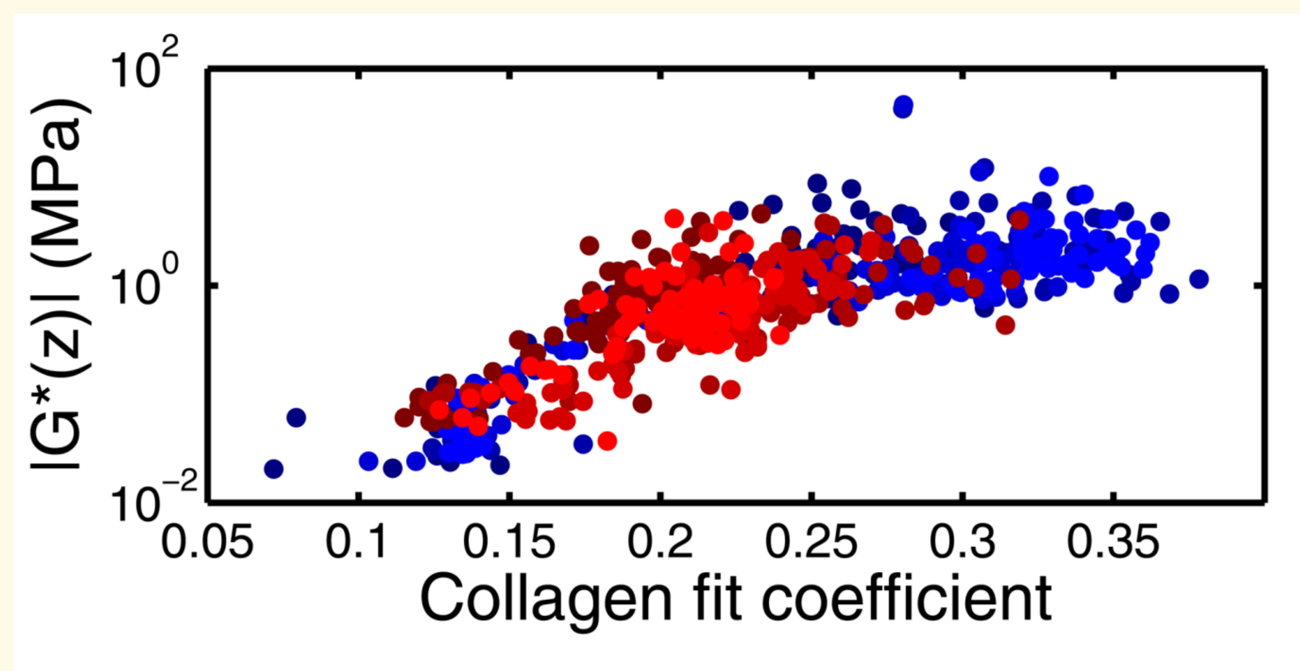
J L Silverberg et al J of Rheology (2012)

Fiber organization NOT correlated with |G*(z)| (?!)
(FTIR measurements)

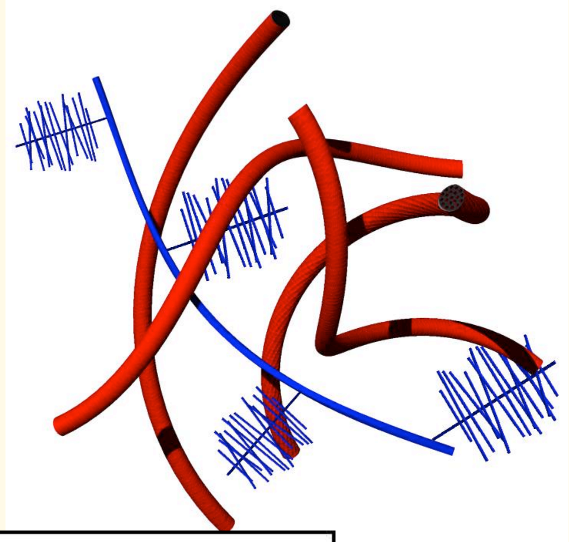


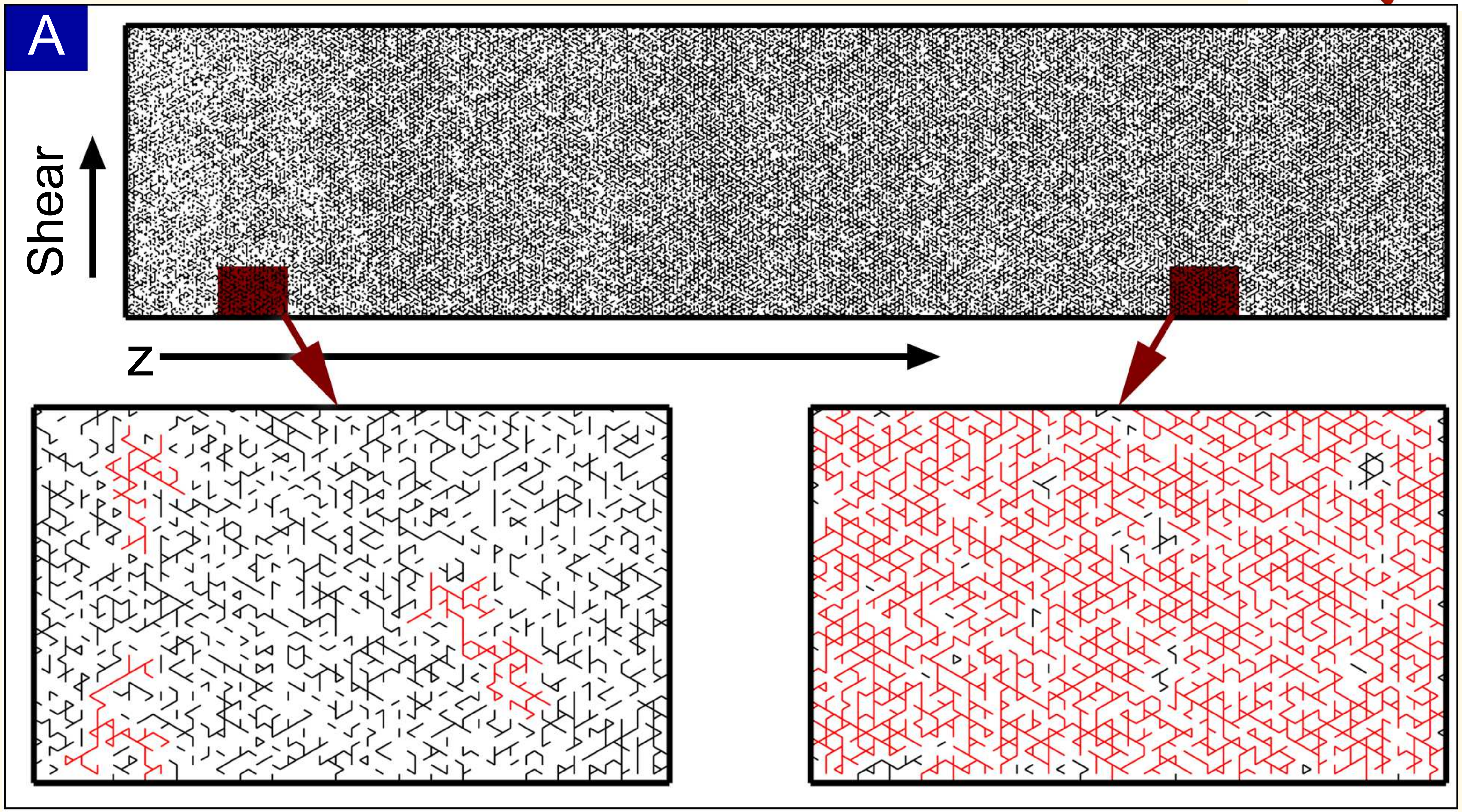
Also... large variations in |G*| with (relatively) small variations in matrix (collagen) density.

How can we explain this?



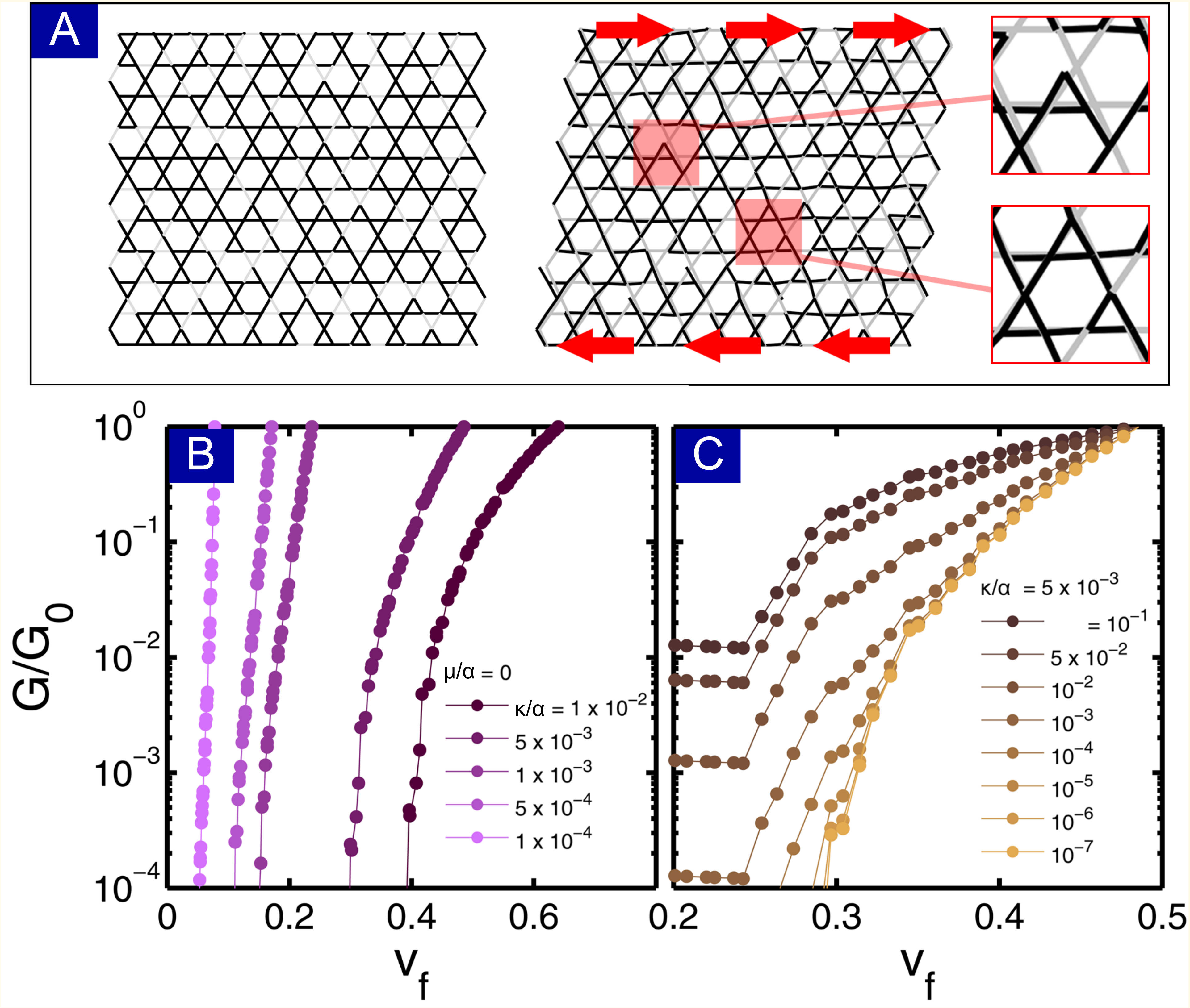
Mechanical response of a composite made of a collagen network and a proteoglycan background



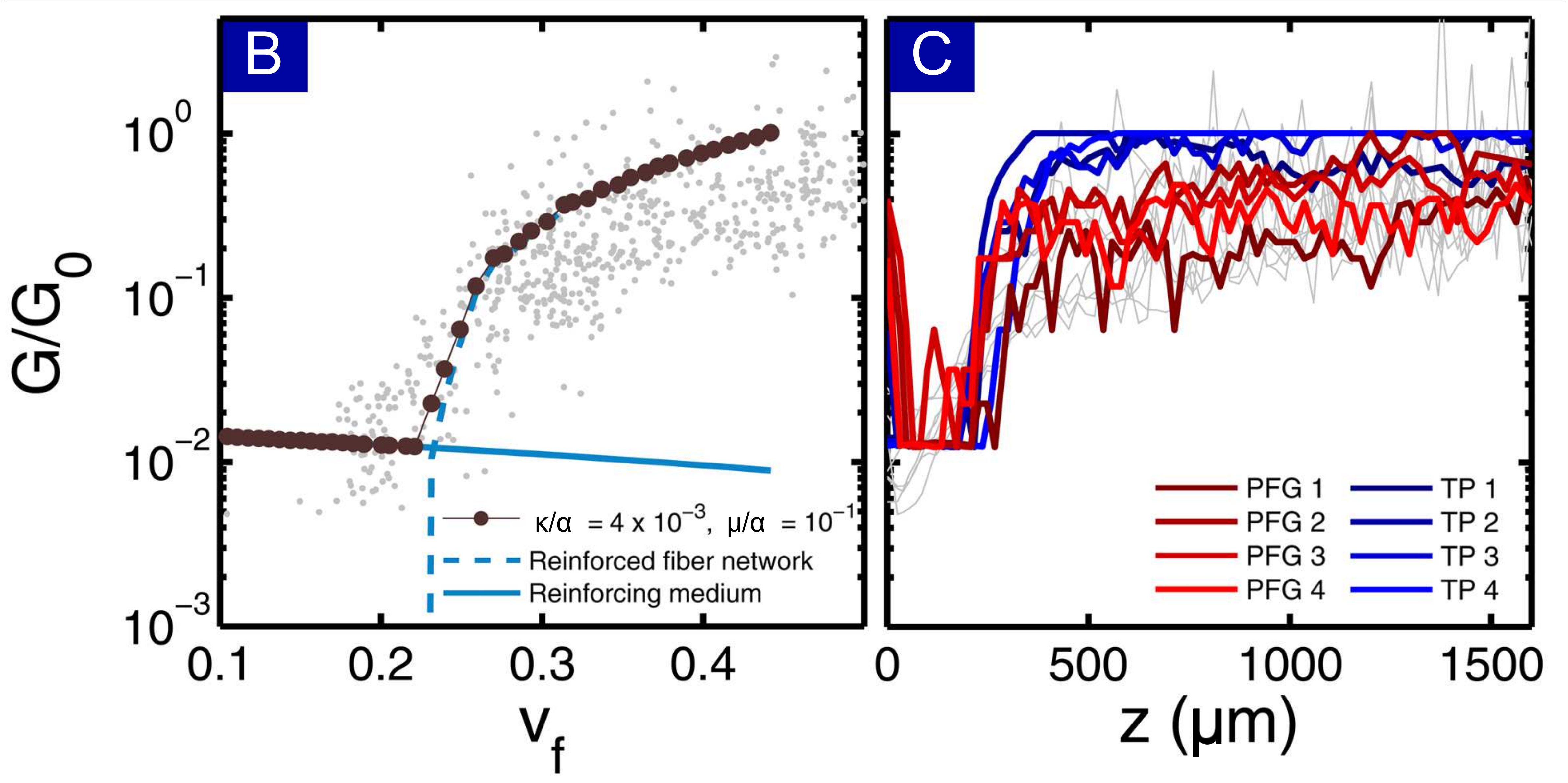


A spatially heterogeneous kagome lattice in a soft elastic background, generated using experimentally measured volume fractions of collagen and proteoglycans at different z.

Shear modulus as a function of volume fraction of collagen and proteoglycans



Depth dependent shear modulus



May provide insights into the microscopic origin of large variations in the simulated depth-dependent G.

Acknowledgements

You !!

F. C. MacKintosh, Vrije Universiteit Amsterdam.

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Students: J. Butcher and J. Shechter

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Past

Ongoing

NWO

vrije Universiteit amsterdam

ROCHESTER INSTITUTE OF TECHNOLOGY R·I·T 1829

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