# Non-Equilibrium Dynamics of Biological Matter and Cellular Mechanosensing


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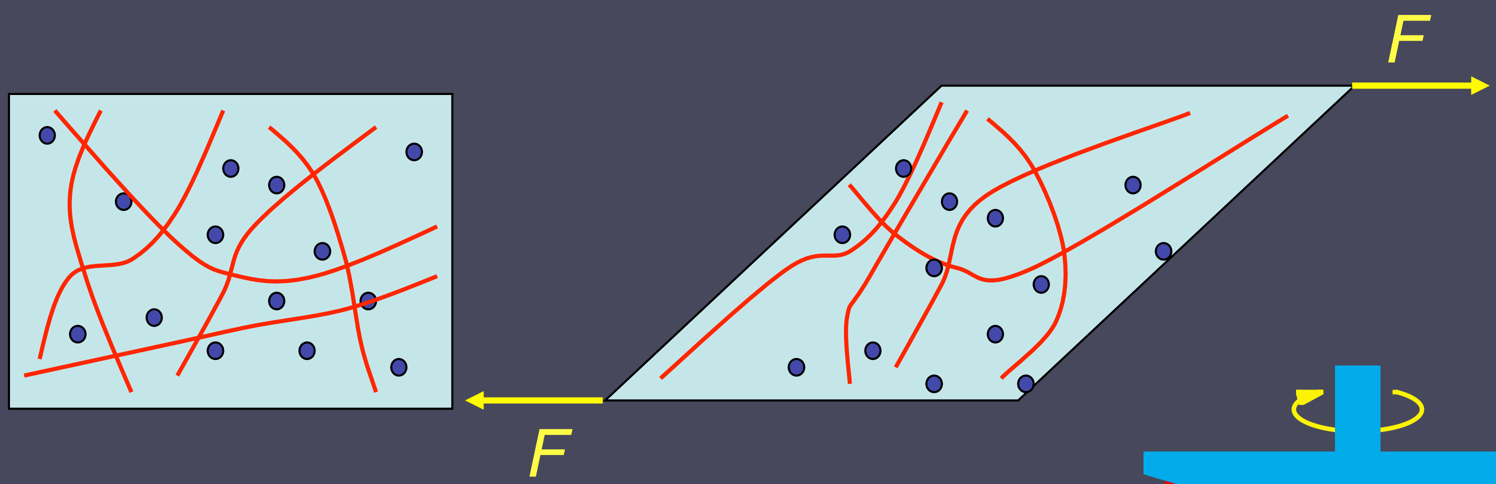
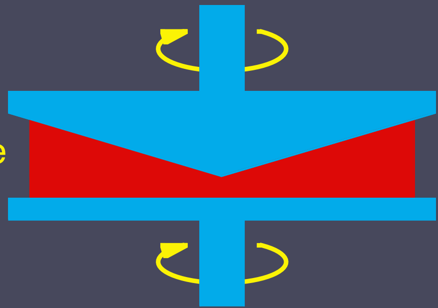
# non-equilibrium mechanics of cells

dancing fish keratocytes

keratocyte waltz, Cyrus Wilson,
Julie Theriot's lab, Stanford

# shear deformation of a viscoelastic object



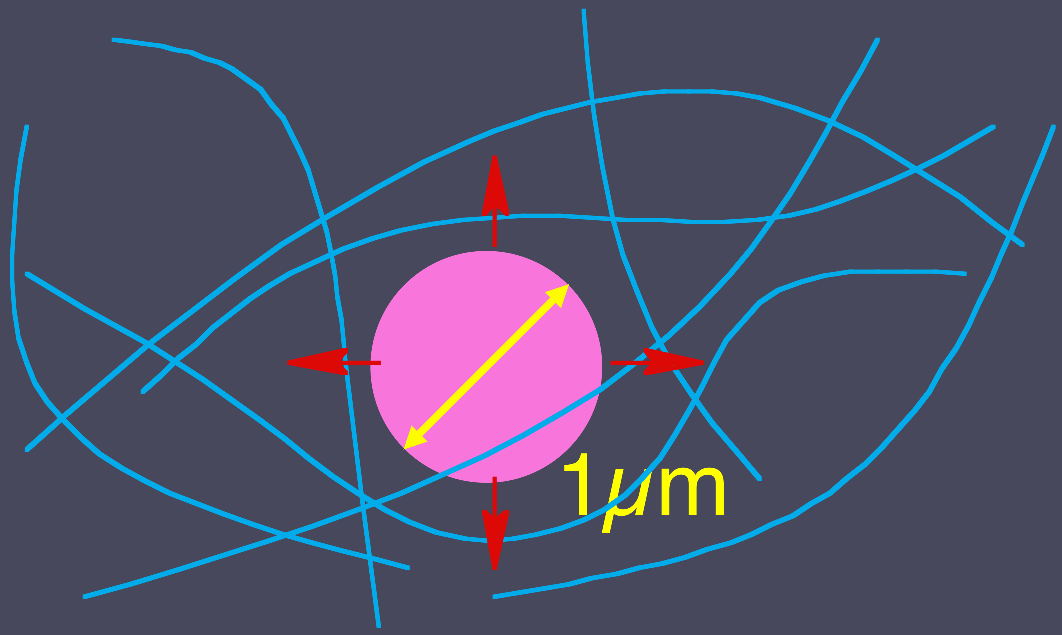




stress = $G$ x strain

complex shear modulus: $G^*(\omega) = G'(\omega) + i\, G''(\omega)$

storage modulus loss modulus

# Microrheology





advantages:

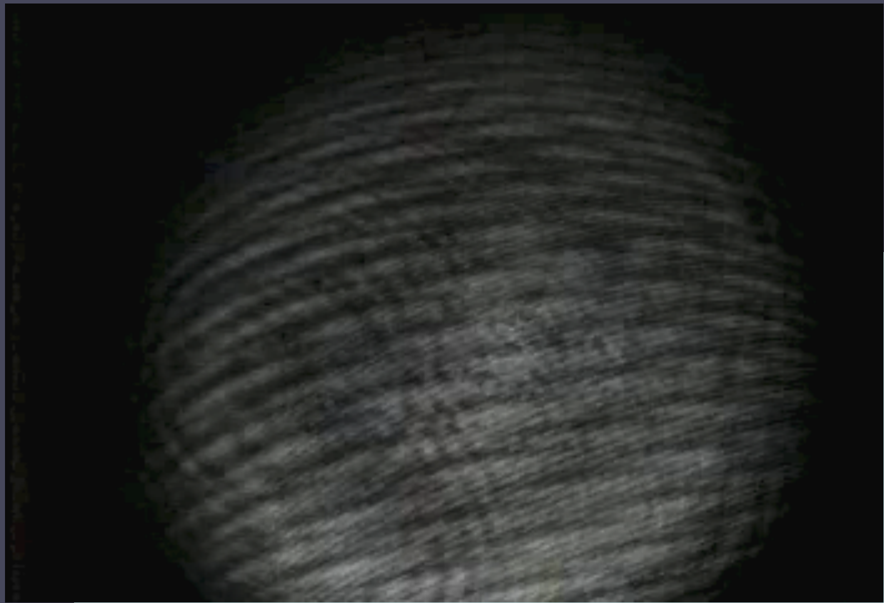
— study inhomogeneities

— study small samples, biological cells

— reach high frequencies (-> MHz) w/o inertial effects

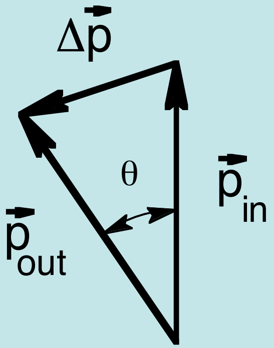
— probe scale dependent material properties by varying probe size

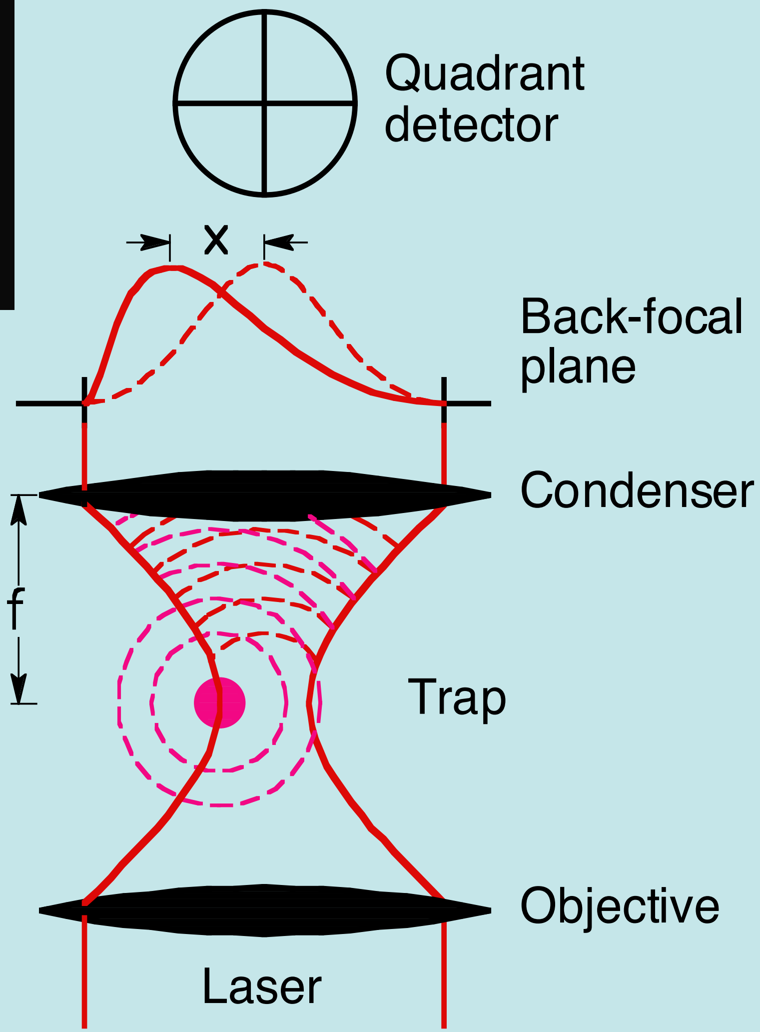
— active vs. passive, single bead vs multiple bead (Sackmann, Valberg, Zaner Fredberg, Weitz & Co.: Mason, Crocker)



# interferometric position detection

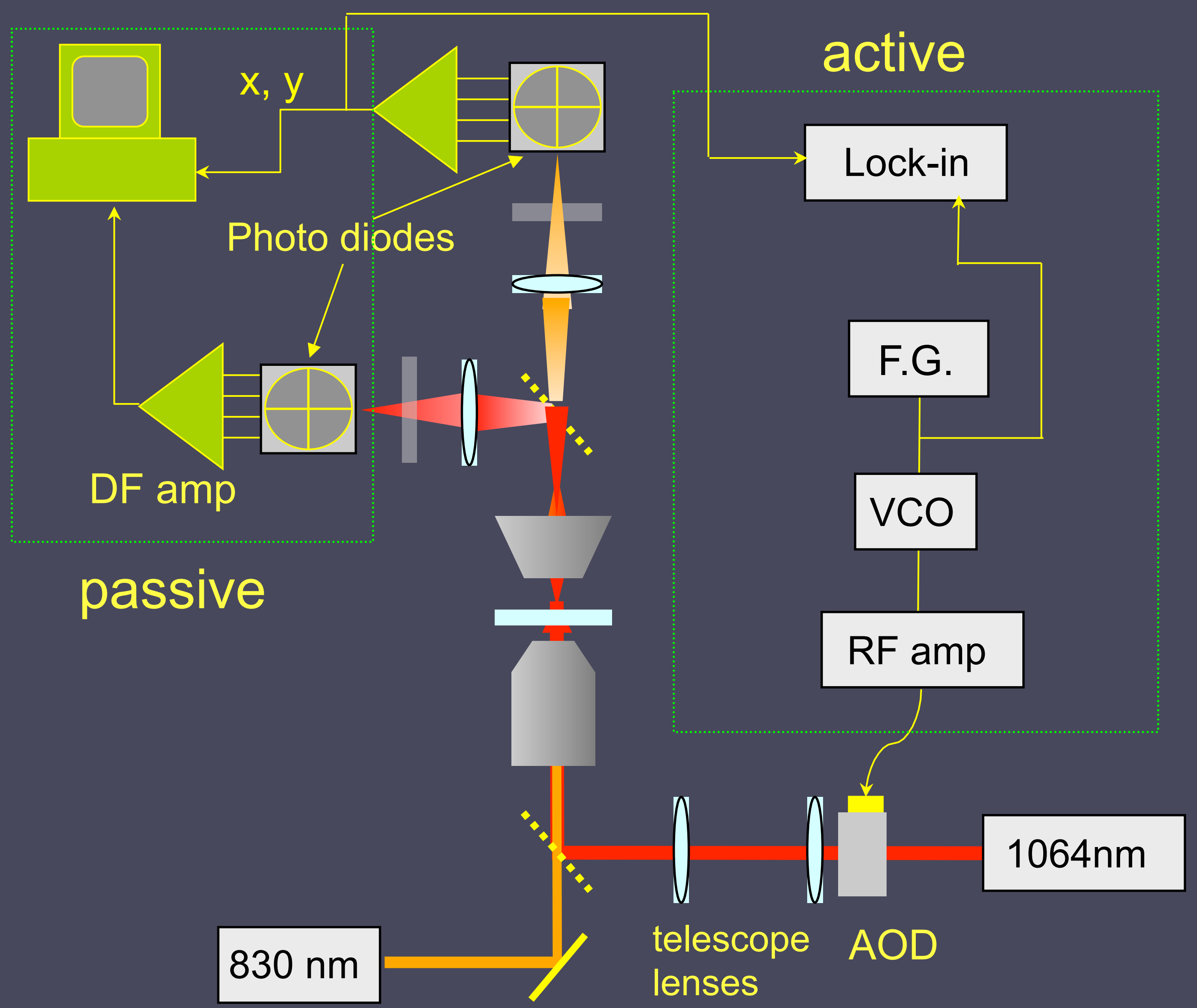
$$F = \frac{dp}{dt} = \frac{I}{c}\sin\theta = \frac{I}{c}\,\frac{x}{f}$$




Gittes, Schmidt, (1998), Opt. Lett. 23: 7-9.

# passive and active microrheology



# passive microrheology, data processing

parameters:

entanglement length > mesh size
persistence length > mesh size
0.1 Hz < $\omega/2\pi$ < 20 kHz

for larger beads and higher frequencies:

generalized Stokes-Einstein:

$$x_\omega = \alpha^*(\omega) f(\omega)$$

$$\alpha^*(\omega) = \frac{1}{6\pi G^*(\omega) a}$$

## data processing:

time-series data, x(t)

power spectral density, $\langle x_\omega^2 \rangle$

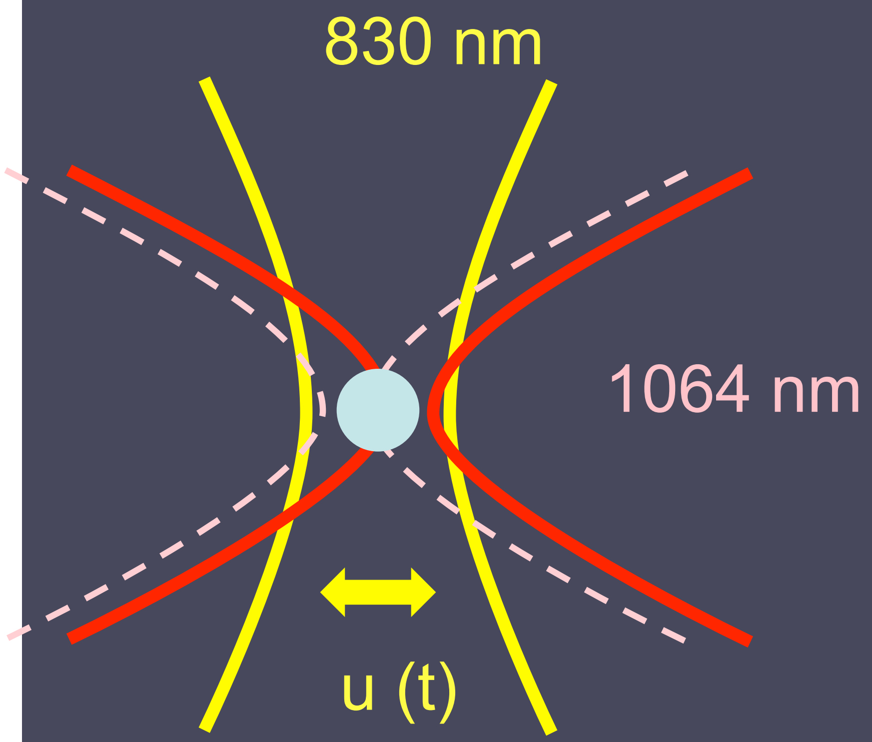
fluctuation-dissipation: $\langle x_\omega^{\ 2} \rangle = \dfrac{4 k_B T \alpha''(\omega)}{\omega}$

Kramers-Kronig: $\alpha'(\omega) = \dfrac{2}{\pi} P \displaystyle\int_0^\infty \frac{\zeta \alpha''(\zeta)}{\zeta^2 - \omega^2}$

$G'(\omega), G''(\omega)$

Gittes, Schnurr, MacKintosh, Schmidt, PRL 79:3286 ('97)
Schnurr, Gittes, MacKintosh, Schmidt, Macromol. 30:7781 ('97)

# active microrheology



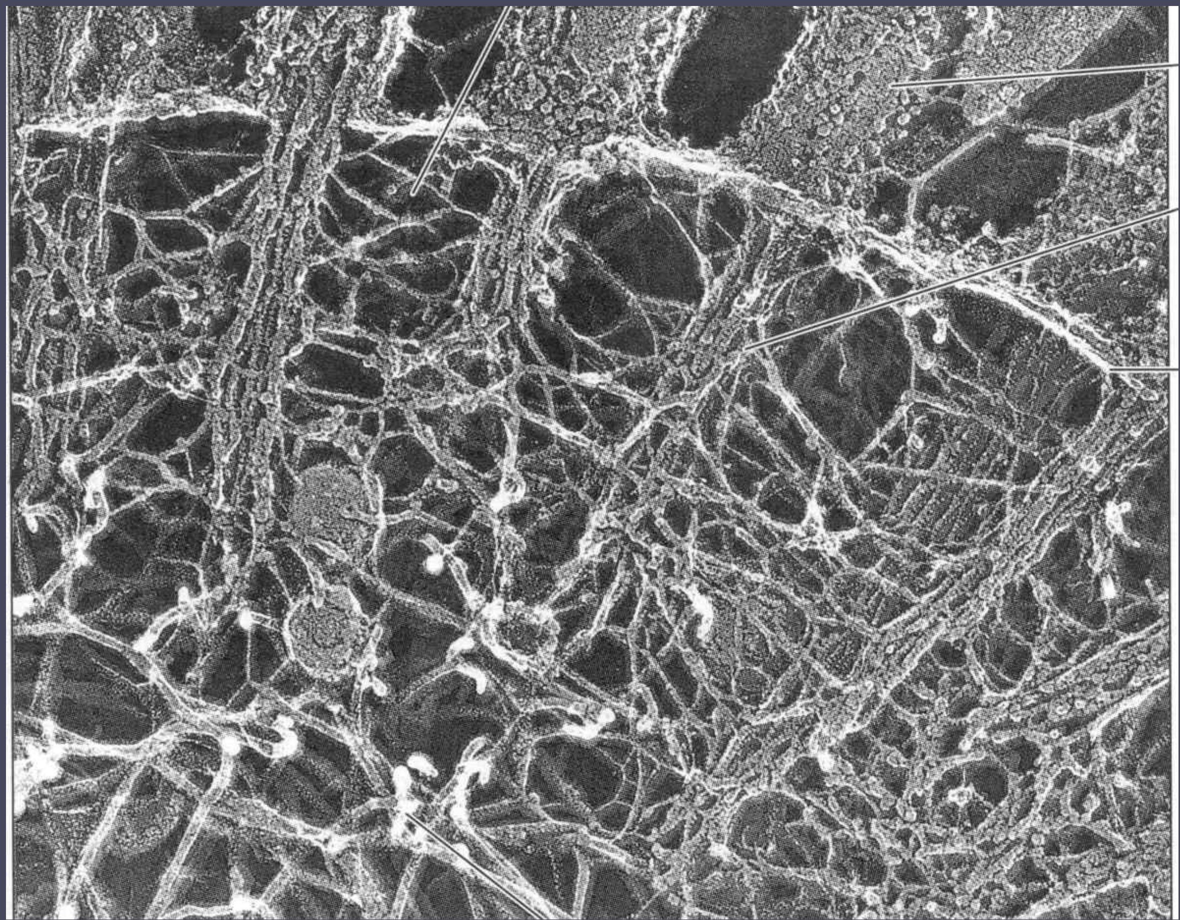

Response function

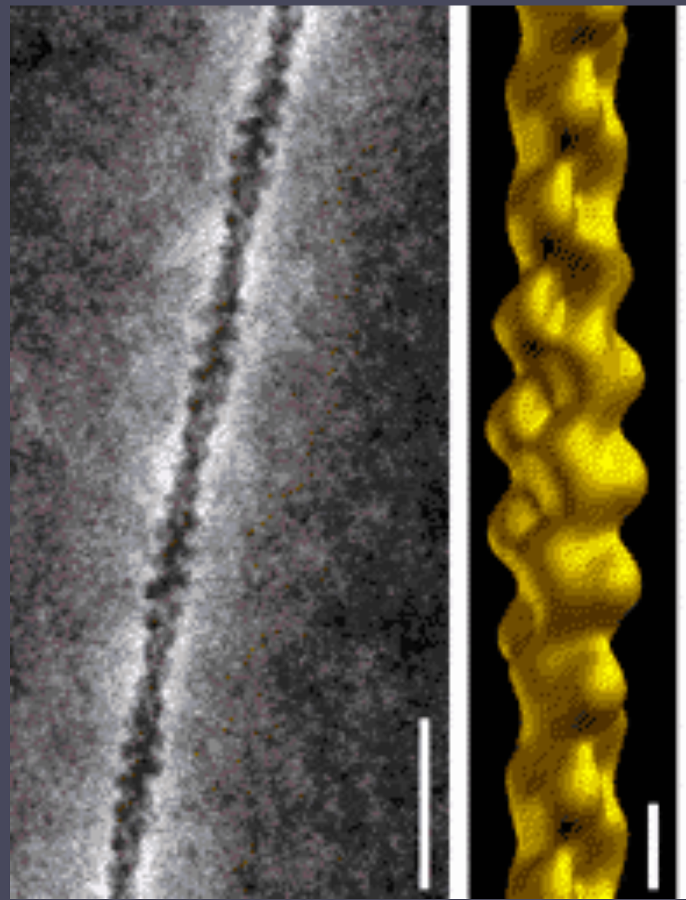
$$u(\omega) = A(\omega)\, F(\omega)$$

Fluctuation-dissipation:

$$C(\omega) = \frac{2k_B T}{\omega} A''(\omega)$$

# biological non-equilibrium network: actin cortex





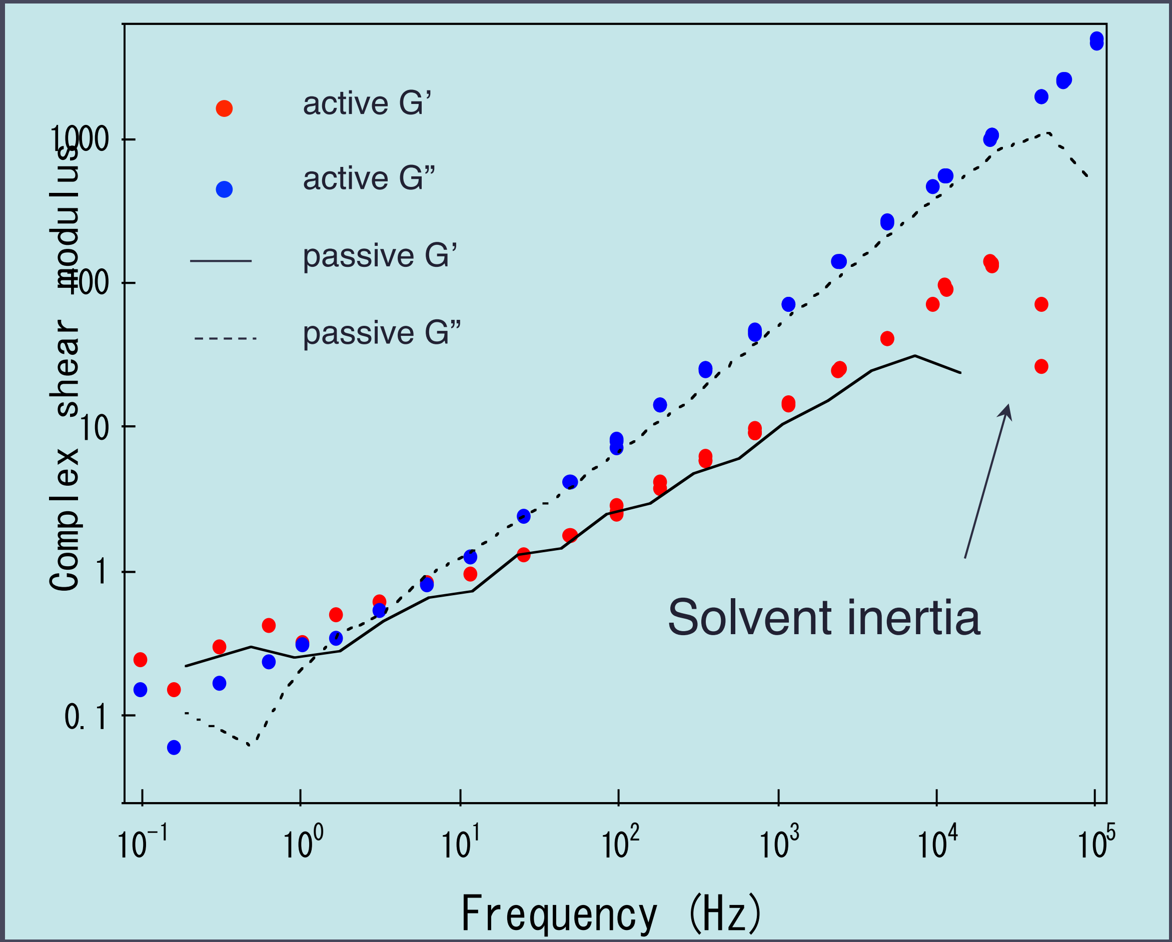
John Heuser, Washington University

# comparison active/passive method

bead size 2.56 $\mu m$

actin solution (1mg/ml)

$$\lim_{\omega \gg \tau_\perp^{-1}} B(\omega) = \frac{\kappa^2}{k_B T}\left(\frac{2i\omega\zeta_\perp}{\kappa}\right)^{3/4}$$



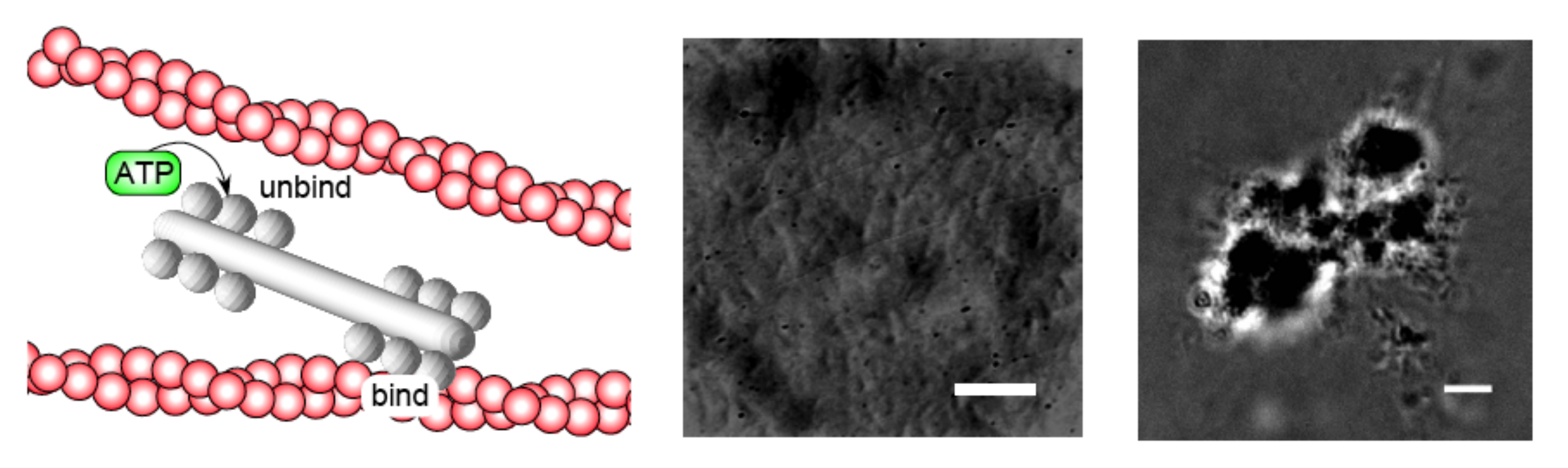

active method : no influence of cut-off in Kramers-Kronig integral, but see solvent inertia

solvent inertia: Atakhorrami, Koenderink, MacKintosh, Schmidt, PRL 95:208302 (2005)

¾ theory: Gittes and MacKintosh: PRE, 58, R1241 (1998); Morse, Macromolecules, 31:7044 (1998)

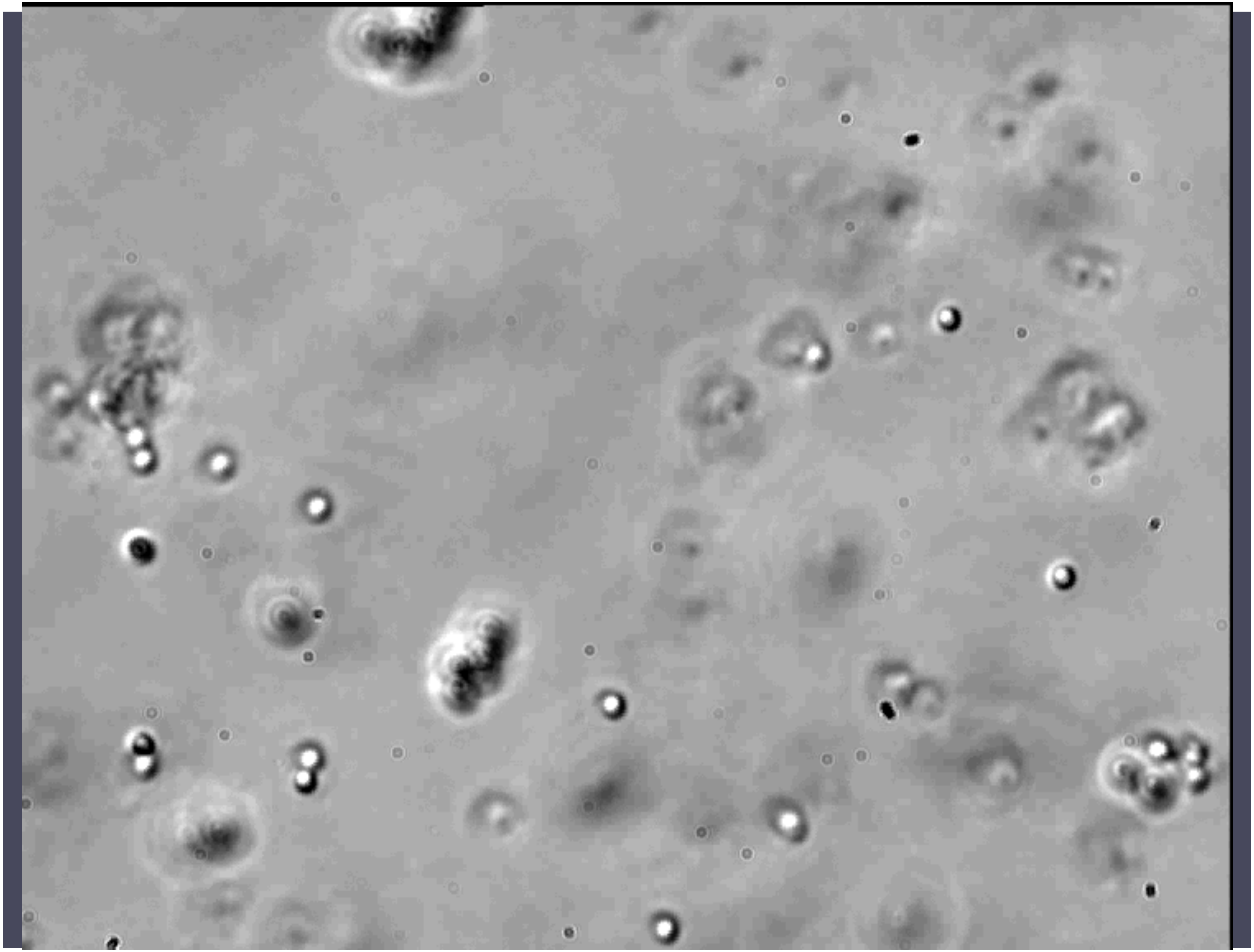
# non-equilibrium model network,

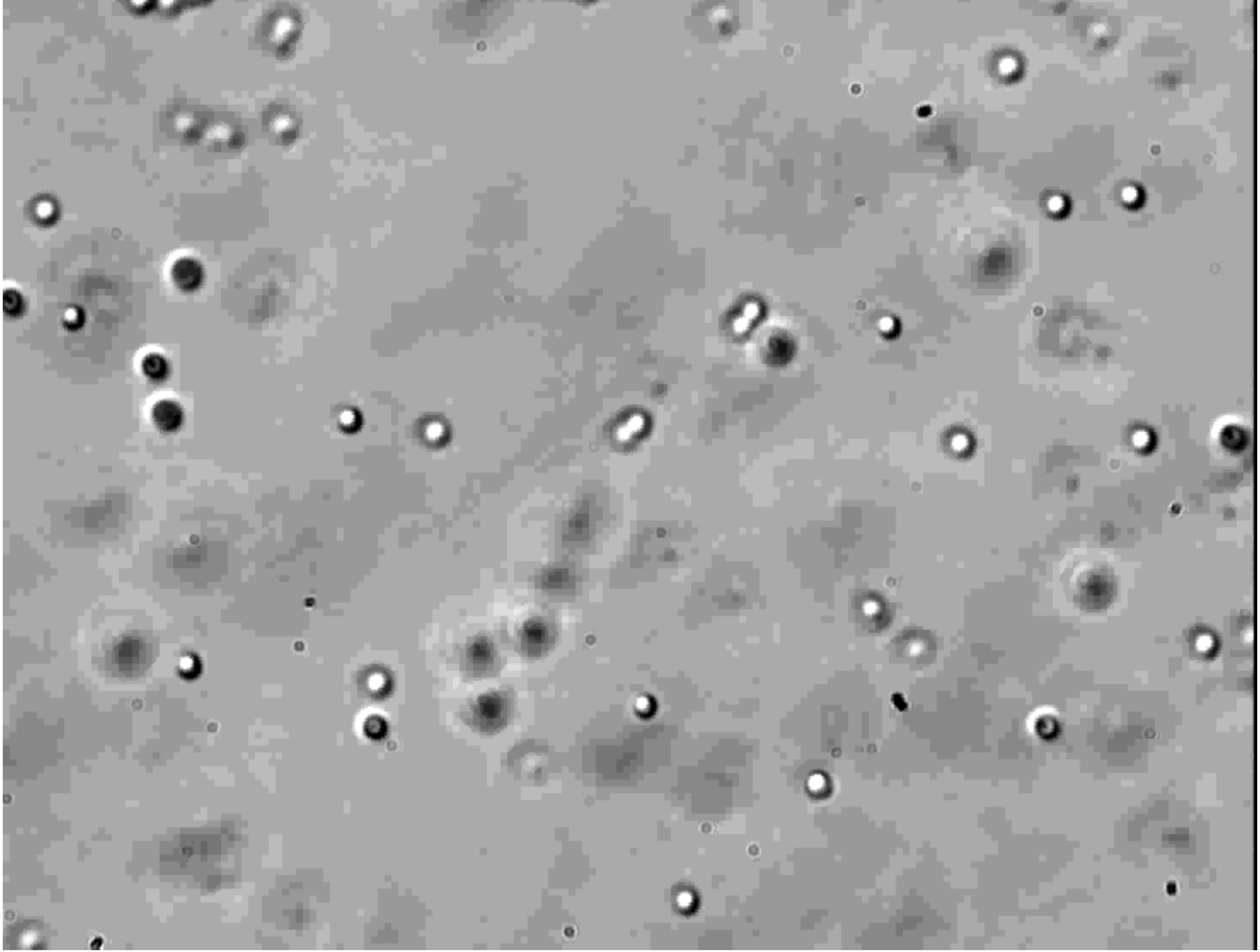
# actin + myosin + cross-linker



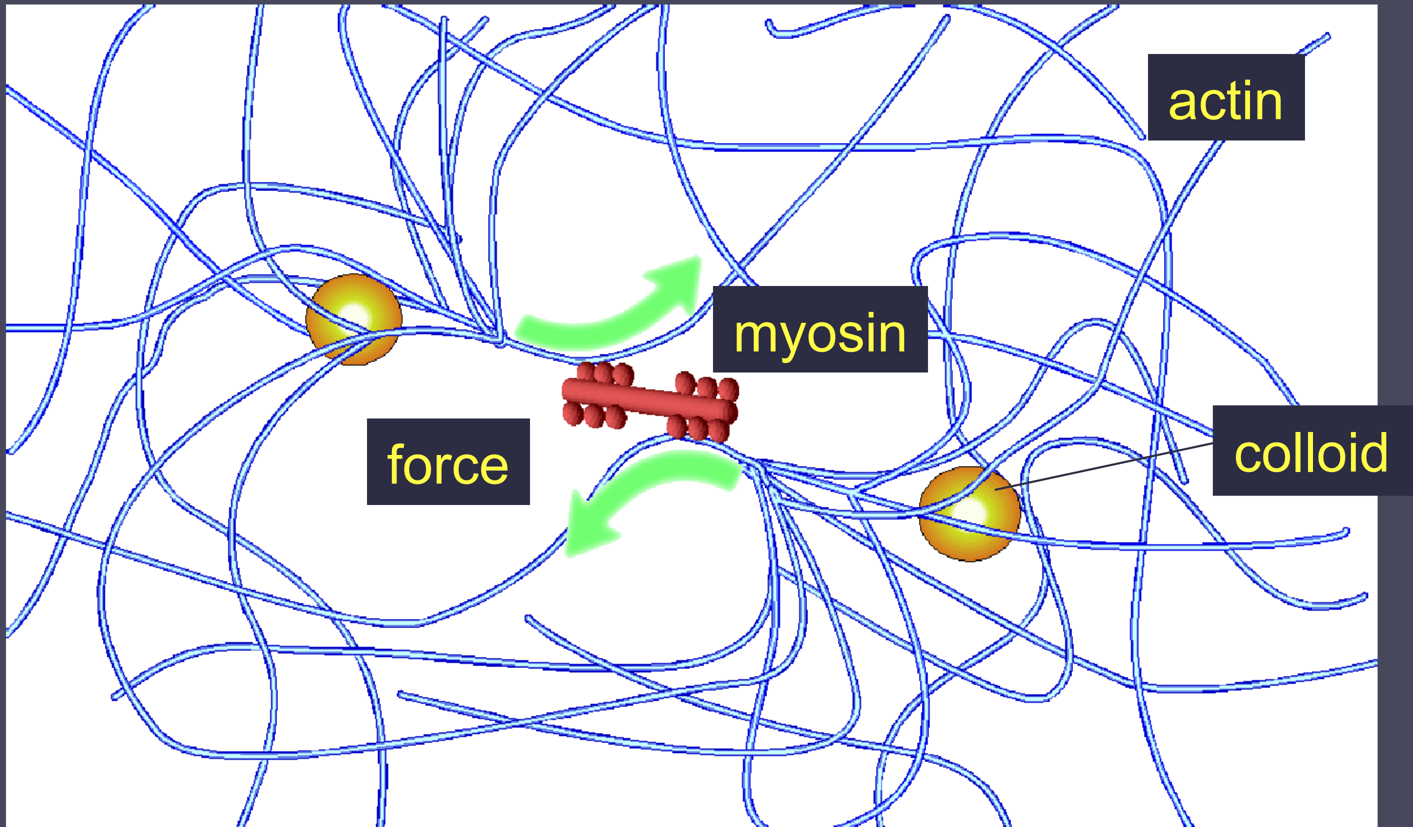

cross-linker: biotin + streptavidin

Mizuno, Tardin, Schmidt, MacKintosh (2007), Science 315, 370





# microrheology in active cytoskeletal networks



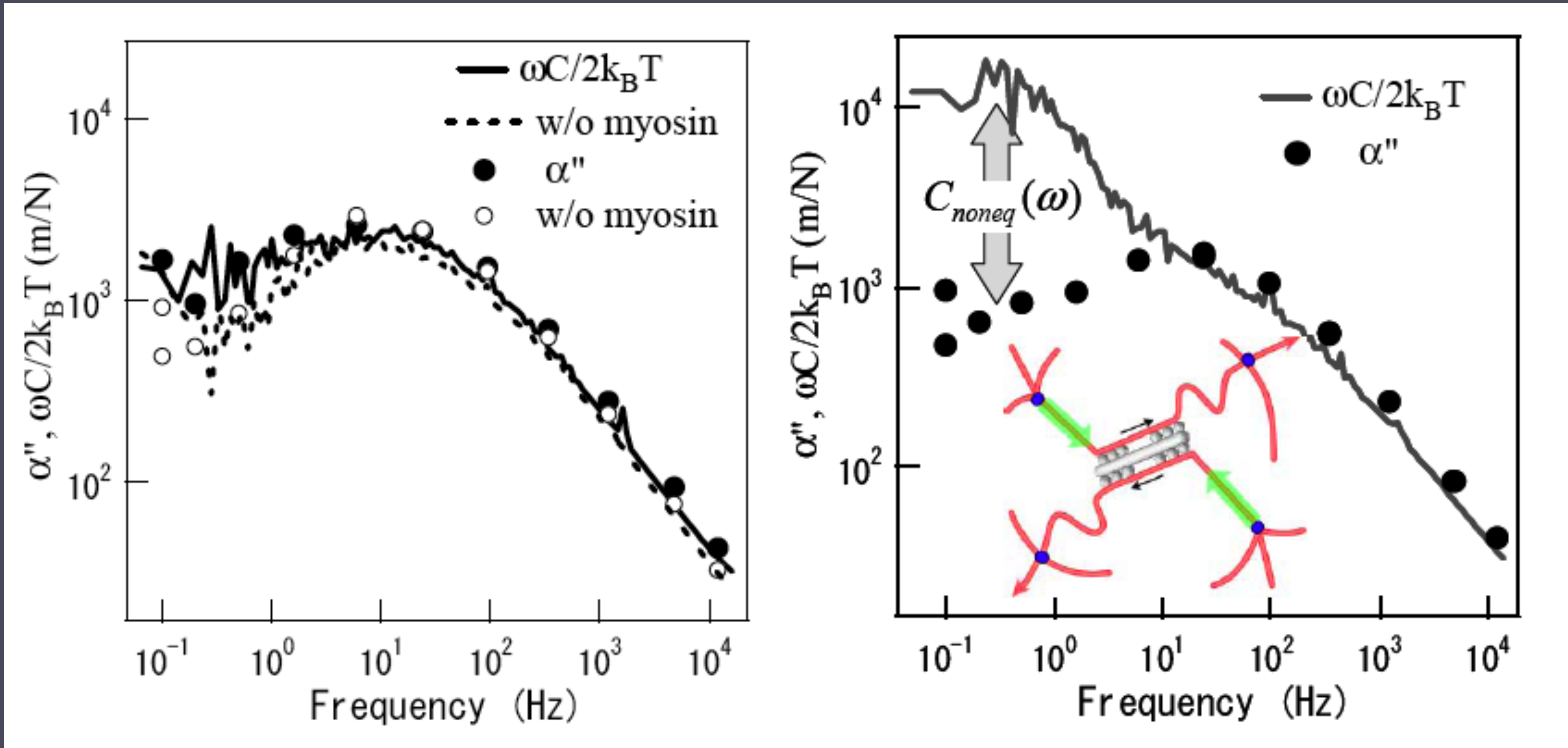

force generation

nonequilibrium fluctuations

mechanical properties

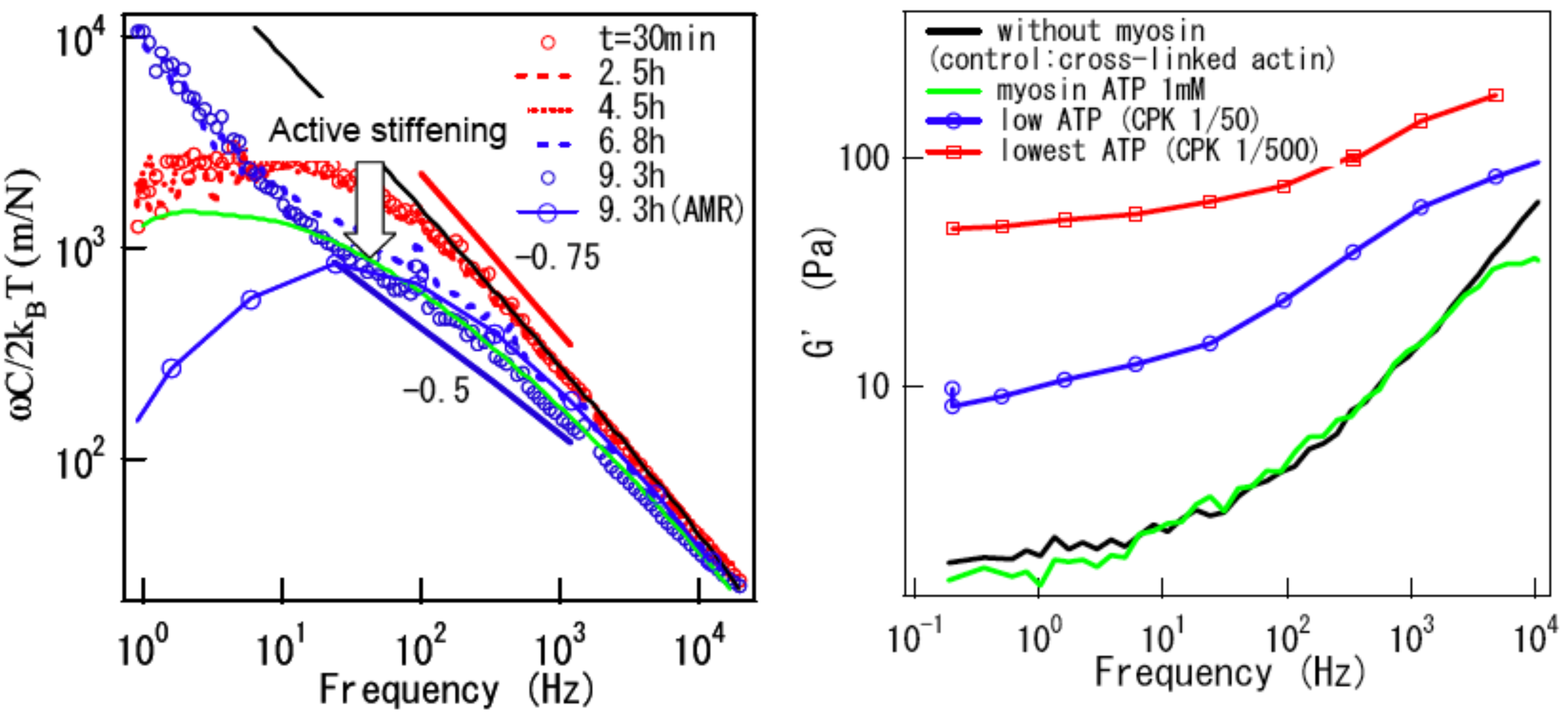
# complex response function, imaginary part

active MR (dots) and passive MR (lines)




fluctuation-dissipation theorem: $\left\langle x_{\omega}{}^{2} \right\rangle = \frac{4k_B T\alpha''(\omega)}{\omega}$

# active stiffening with increasing motor-generated tension



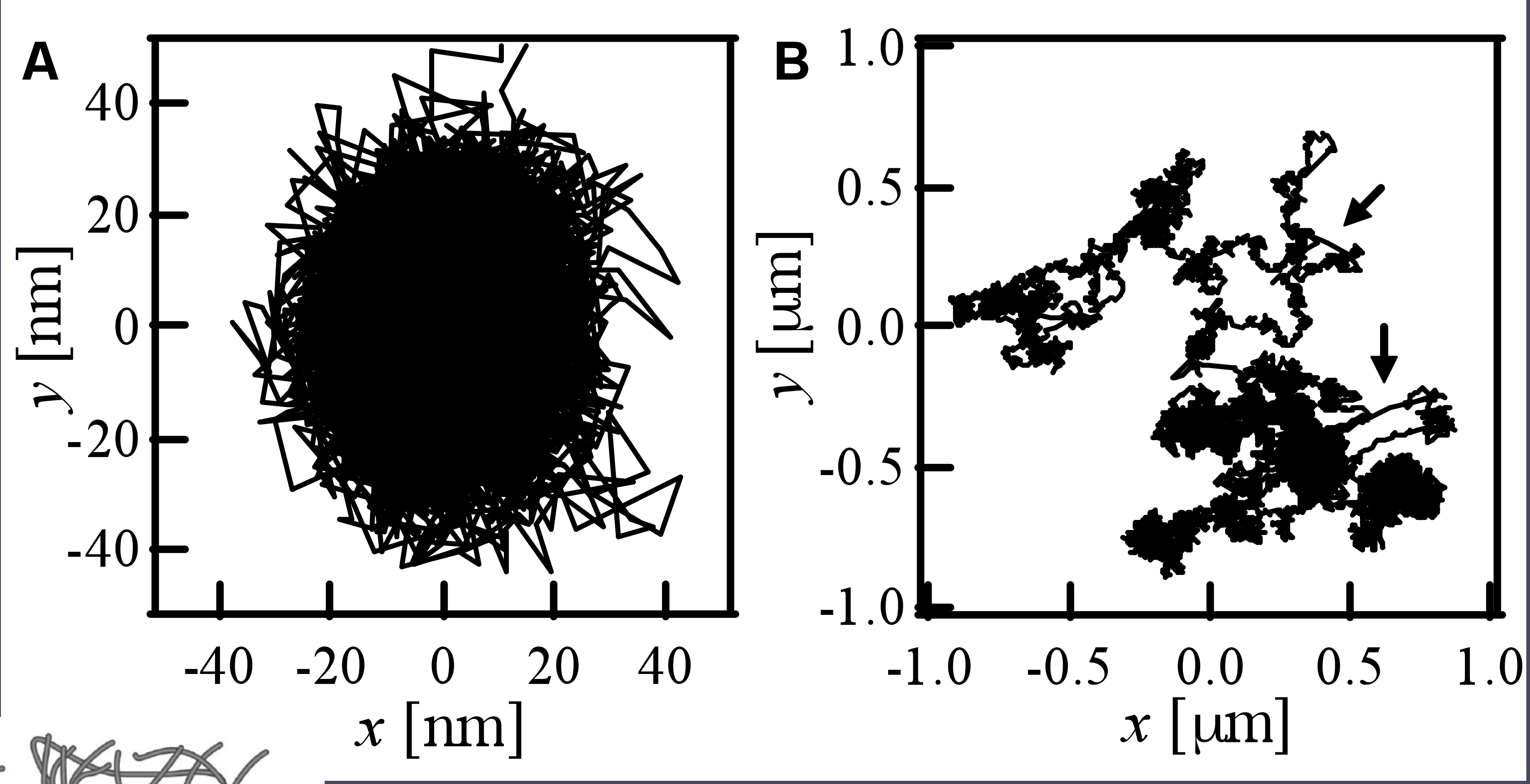

$$\alpha = \frac{5}{2\pi a \rho l_c} \sum_q \frac{2k_B T q^4}{\omega_q \zeta^2 (2\omega_q - i\omega)}$$

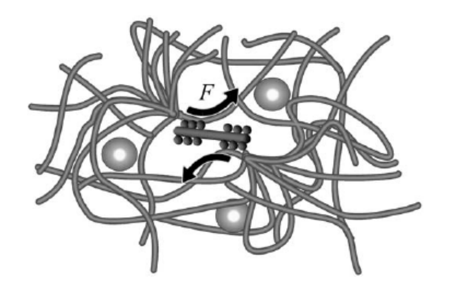
$$\omega_q = (\sigma q^2 + \kappa q^4)/\zeta$$

$l_c = 2.6\ \mu m, \quad \sigma \sim 1$ pN

# model network: particle trajectories, video microscopy

equilibrium: no myosin　　　　active: with myosin



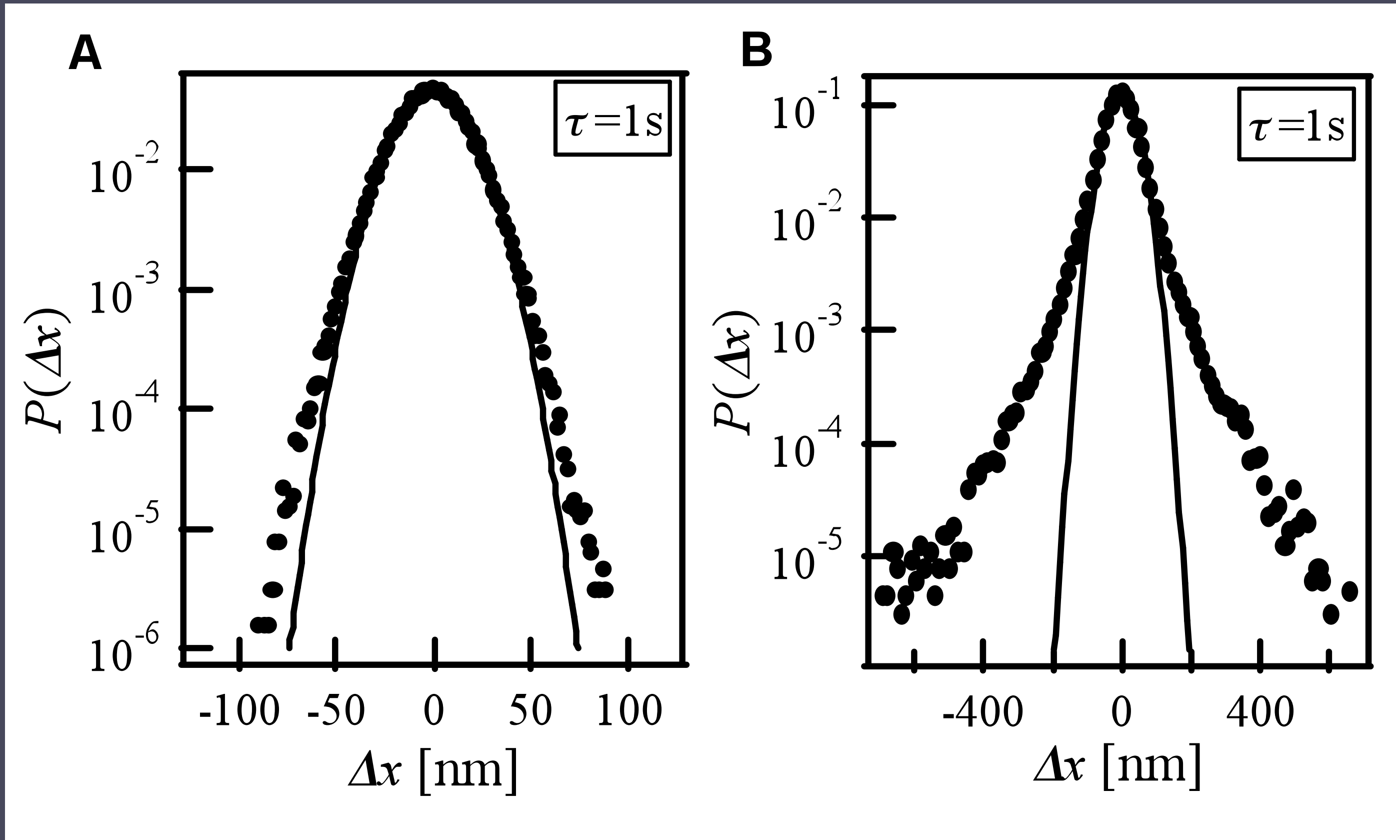




1 µm beads, actin + myosin + cross linker

Toyota, Head, Schmidt, Mizuno, Soft Matter (2011) 7: 3234

# van Hove displacement correlation functions

equilibrium: no myosin active: with myosin

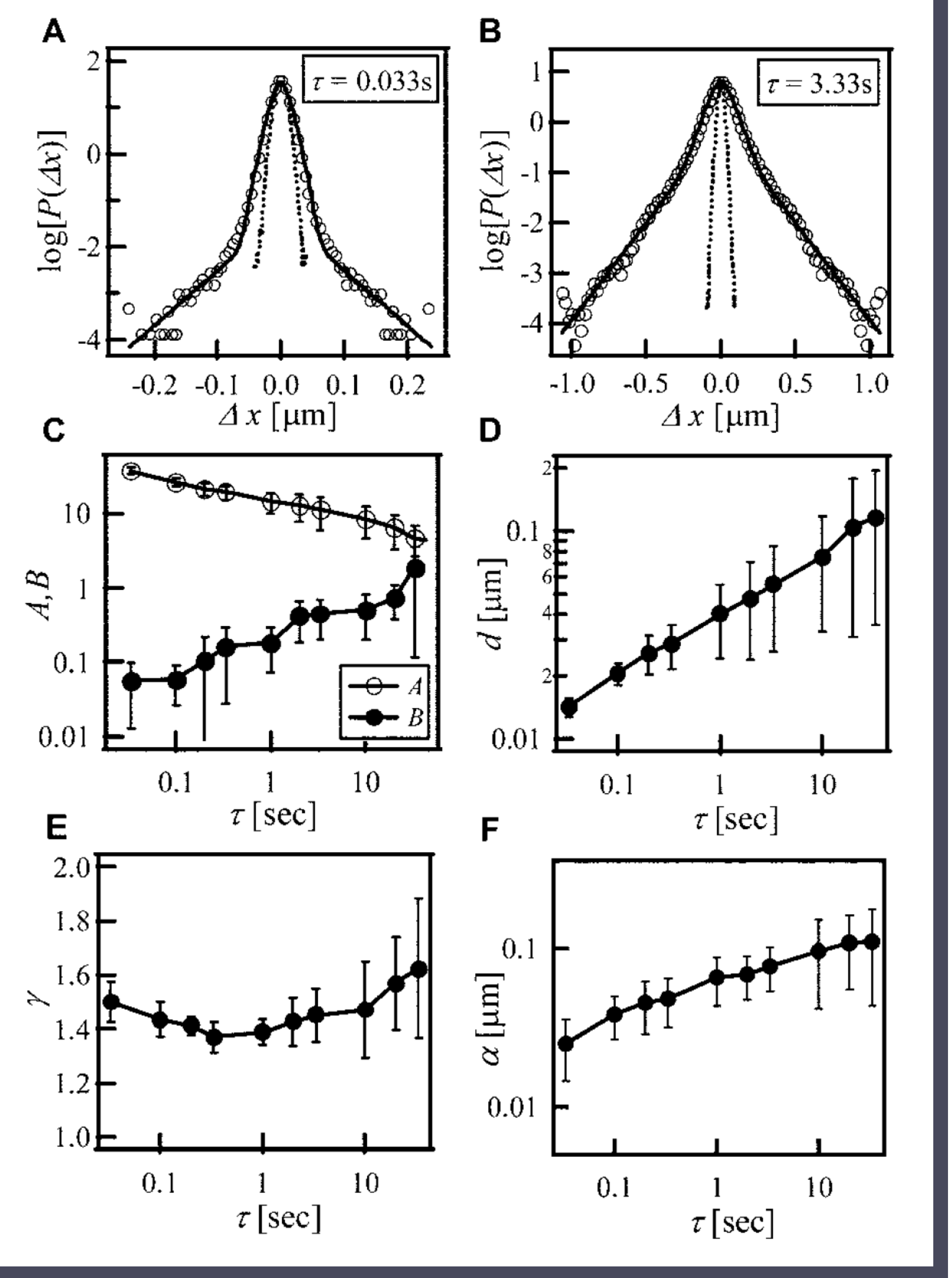



— Gaussian

$$P(\Delta x(\tau)),\ \Delta x(\tau) = x(t+\tau) - x(t)$$

fit with sum of
Gaussian and exponential:

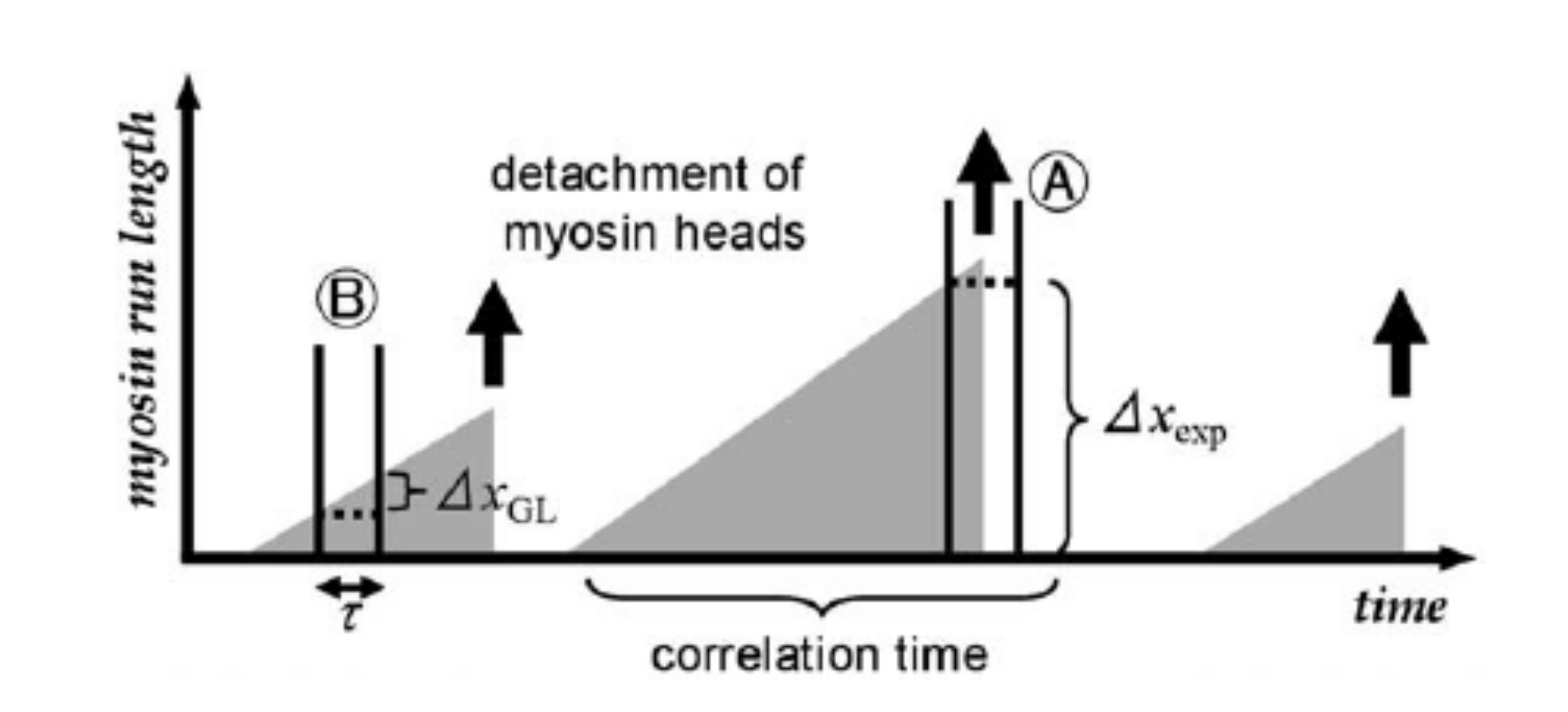
$$f(x) = A\exp[-(x/d)^{\gamma}] + B\exp[-x/\alpha]$$




Gaussian:
many motors far away
⇔ short distances

exponential:
single closest motor, Poisson statistics
⇔ long distances

# time dependence of force generation



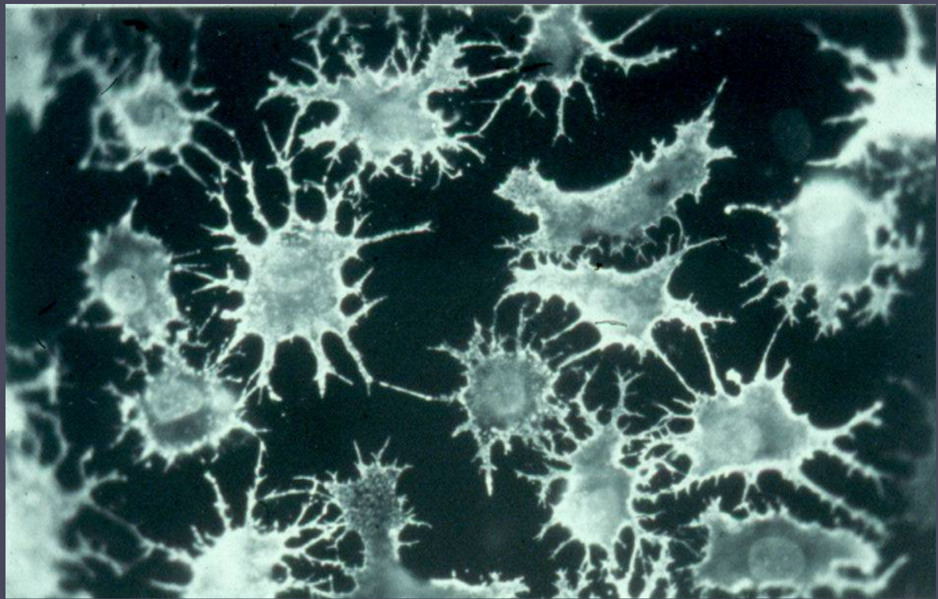

random release => Poisson process, exponential distr.

See also: Zaid, Dunkel, Yeomans: Levy fluctuations and mixing in dilute suspensions of algae and bacteria, Interface online 2-2011

# cells

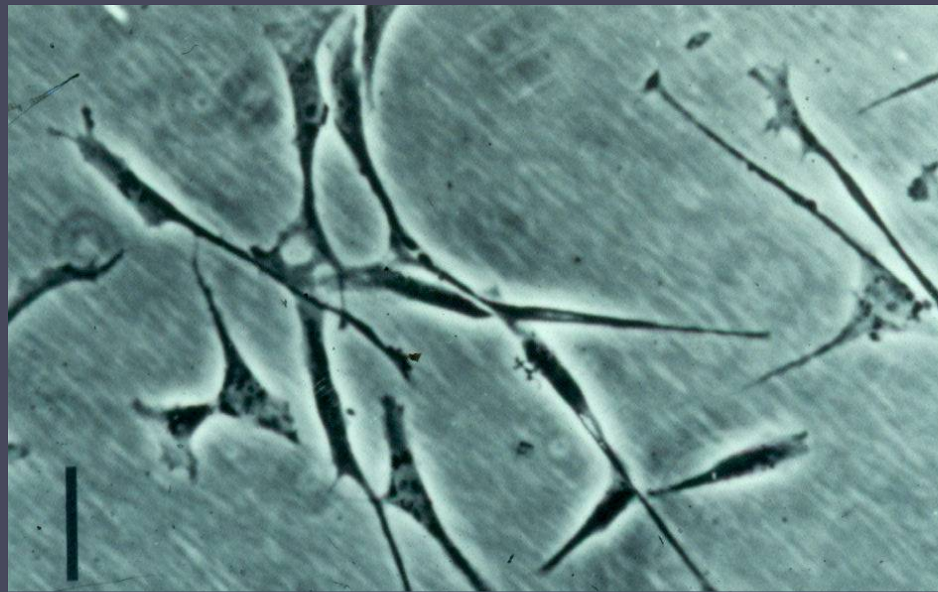
## osteocytes

- MLO-Y4
- Mechanosensory cells embedded in compact bone



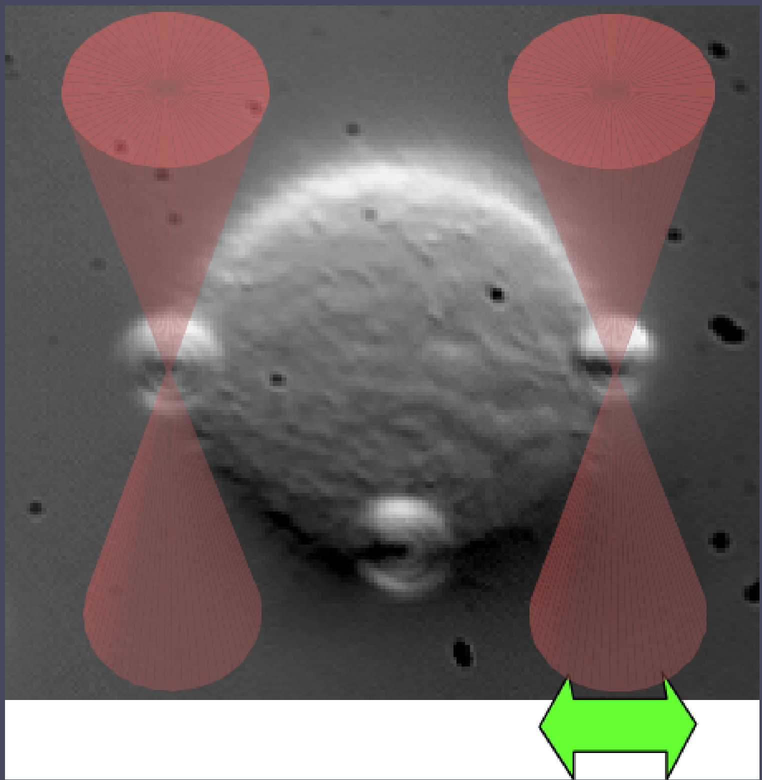
## fibroblasts

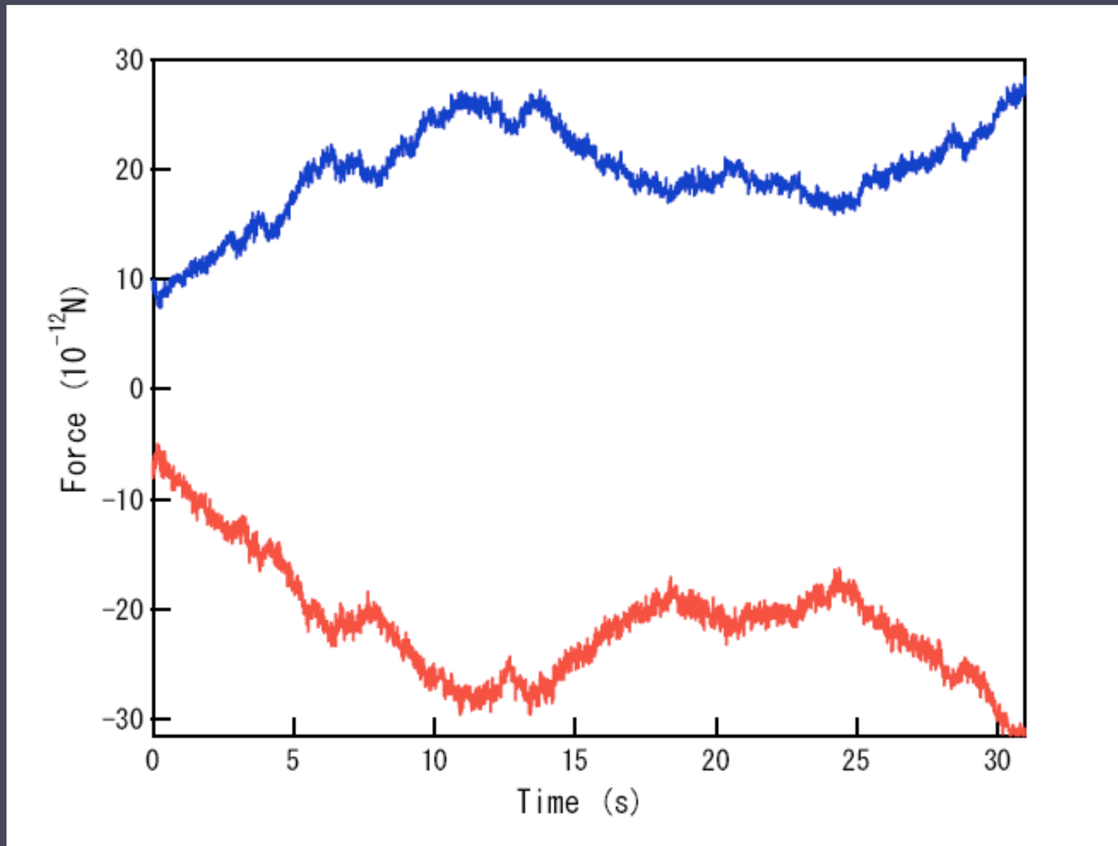
- 3T3 fibroblasts
- Mechanoresponsive cells in connective tissue



# probing cells with two optical traps

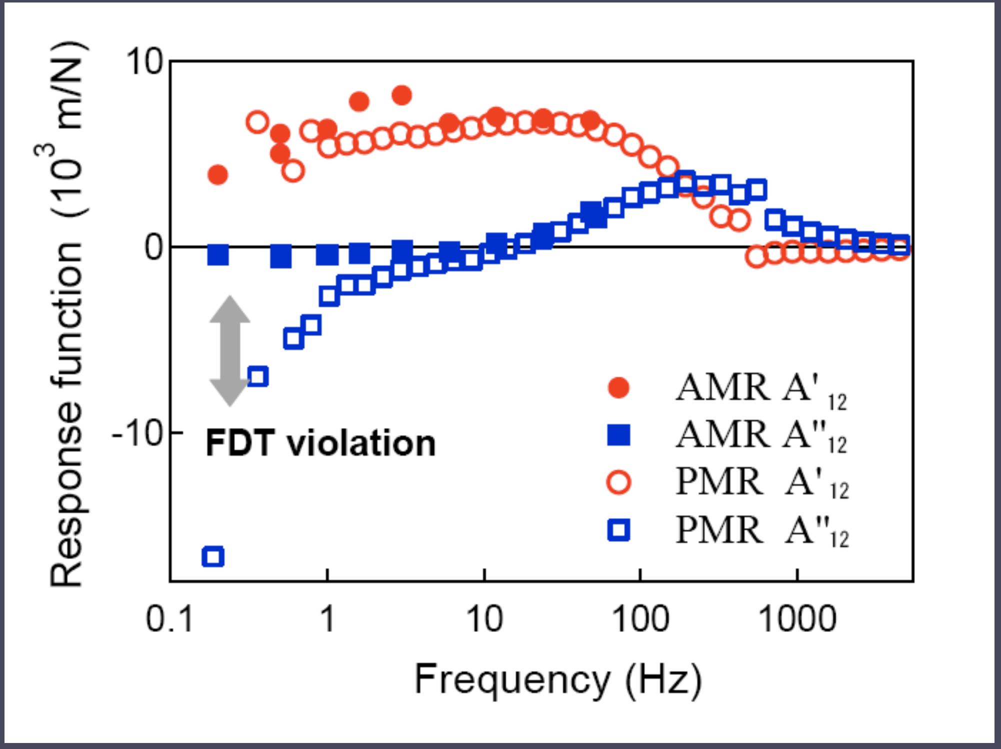
# anti-correlated fluctuations of a trapped cell



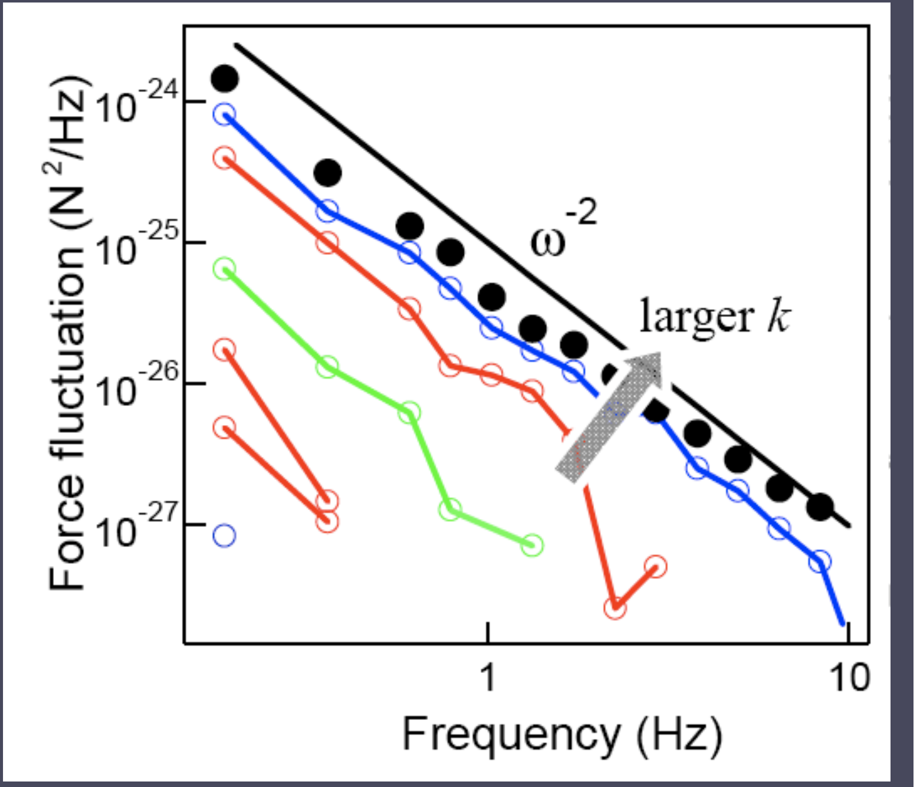


# spectrum of anti-correlated fluctuations of a trapped cell

response function




force fluctuation spectrum



# non-equilibrium part of fluctuations from active and passive measurement

from AMR

FDT

$$P_{th}(\omega) = 2k_B T A''_{ij}/\omega$$

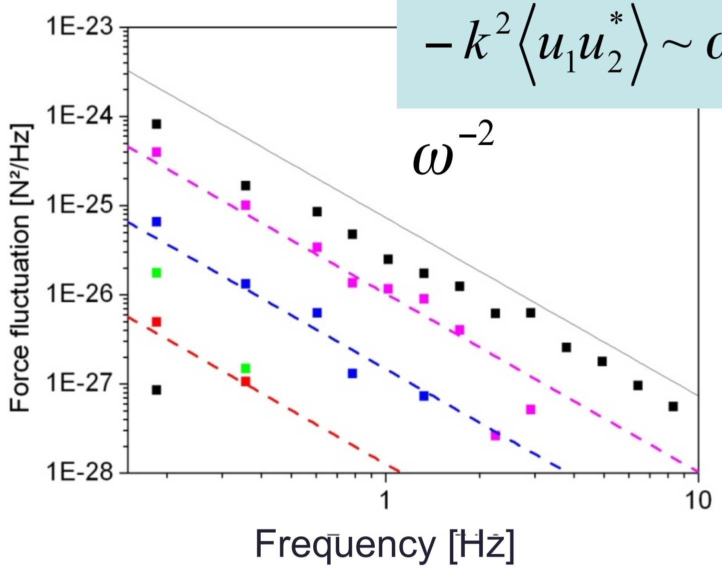
from PMR

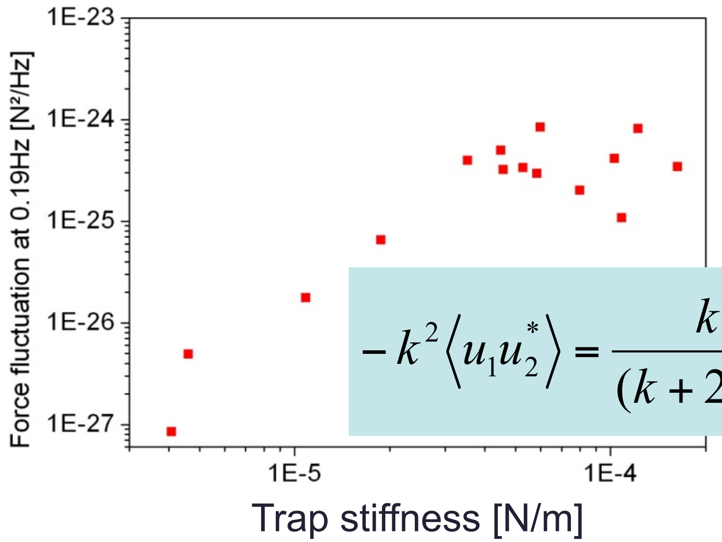
$$P_{act}(\omega) = \langle u_i u_j^* \rangle - 2k_B T A''_{ij}/\omega$$

# force fluctuations

## osteocytes

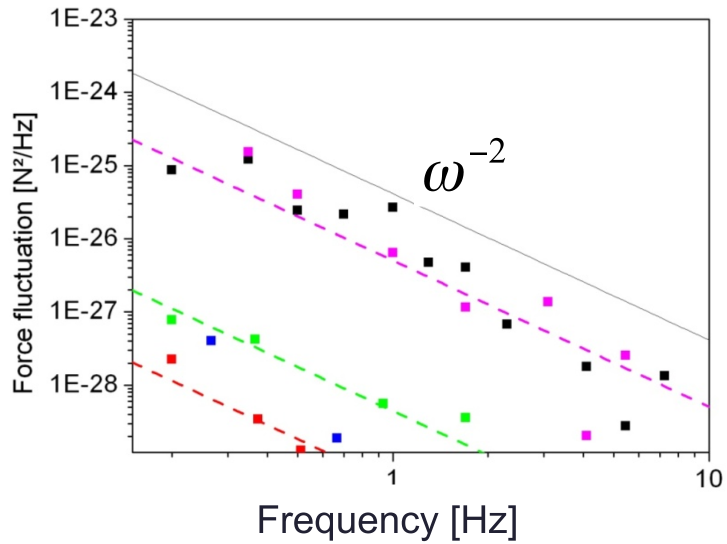
$$-k^2\langle u_1u_2^*\rangle \sim \omega^{-2}$$

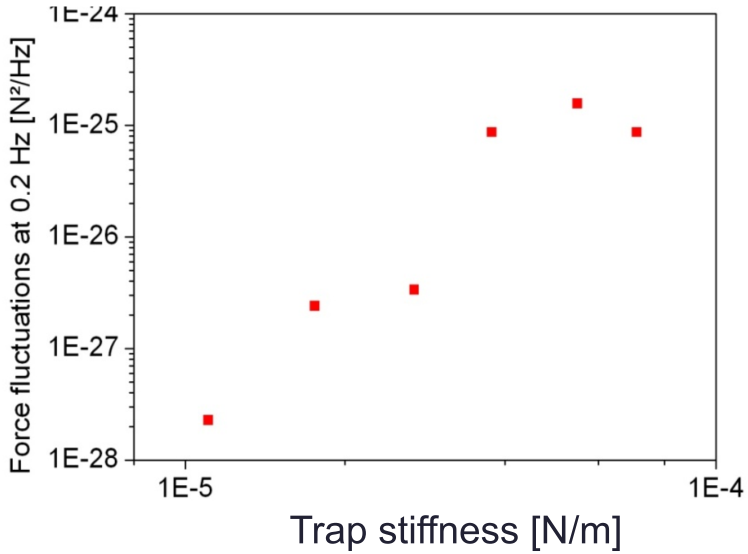


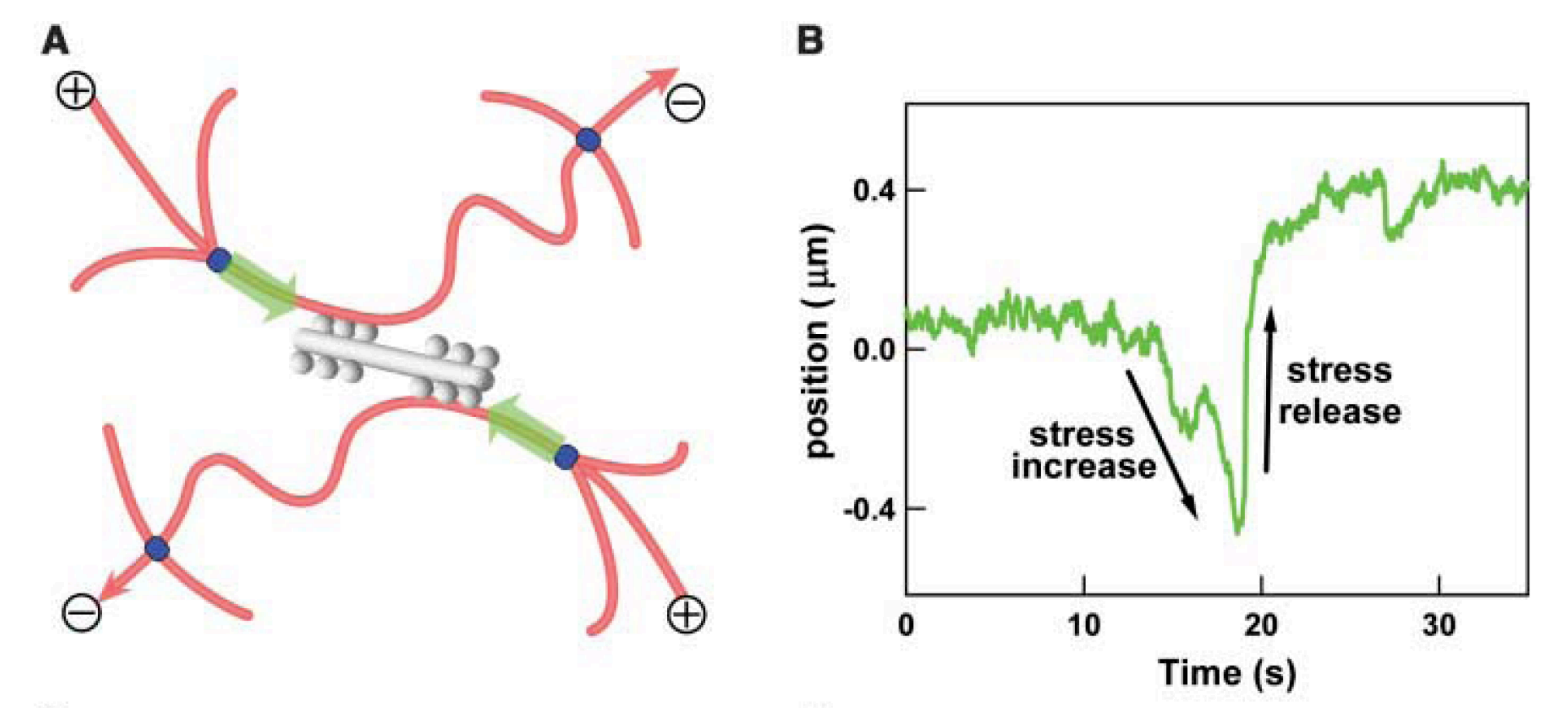

$$-k^2\langle u_1u_2^*\rangle = \frac{k^2}{(k+2k_{12})^2}\langle FF^*\rangle$$

## fibroblasts

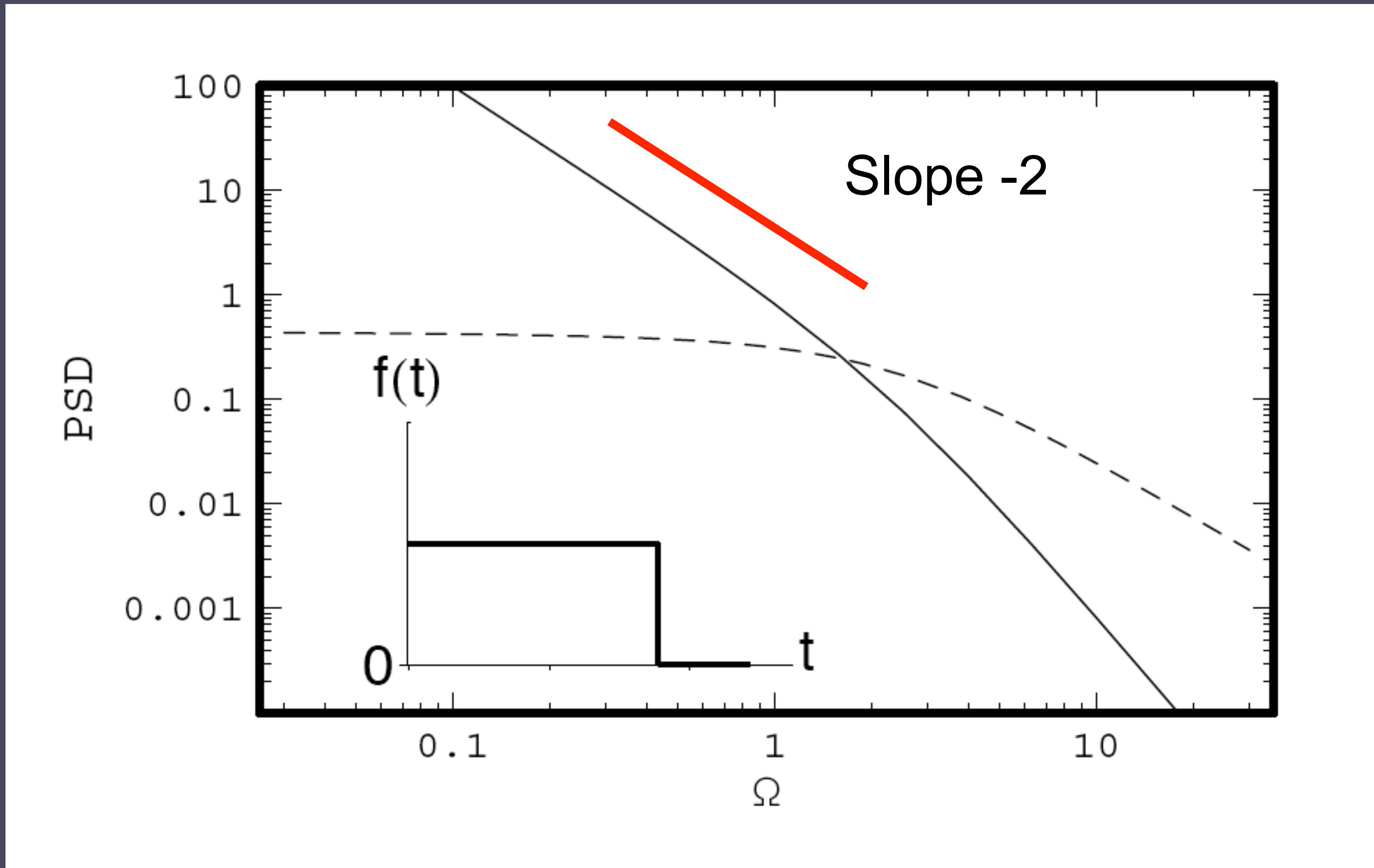


# motors generate contractile stress/strain: slow build-up, sudden (catastrophic) release



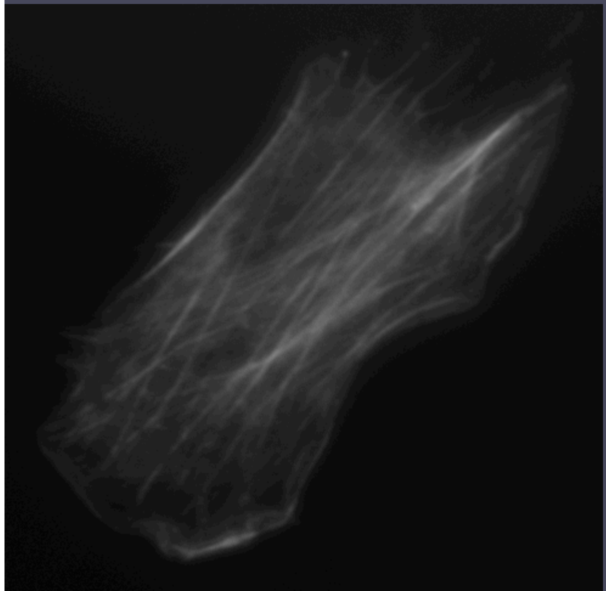
# PSD slope due to active fluctuations

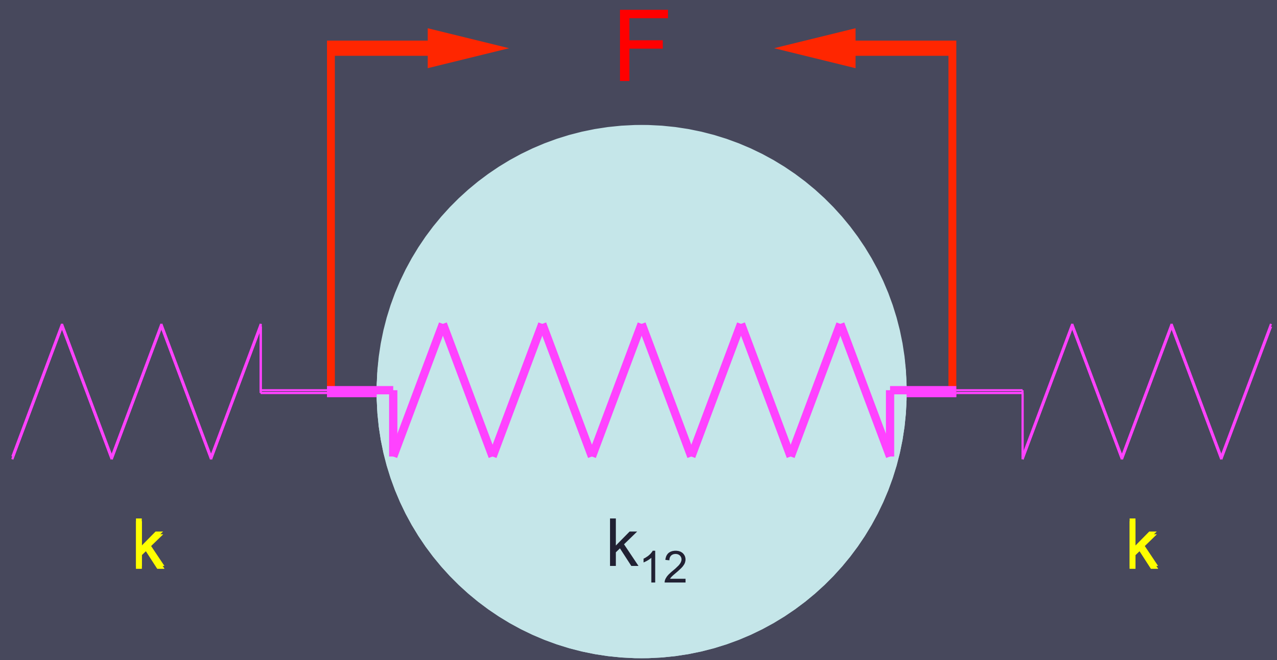



$$\langle |u(\omega)|^2 \rangle \propto \langle |f(\omega)|^2 \rangle / |G(\omega)|^2 \propto |\omega G|^{-2}$$

Levine, MacKintosh, PRL 2008, J Phys Chem, 113: 3820 (2009)

# from displacement fluctuations in traps to force fluctuations



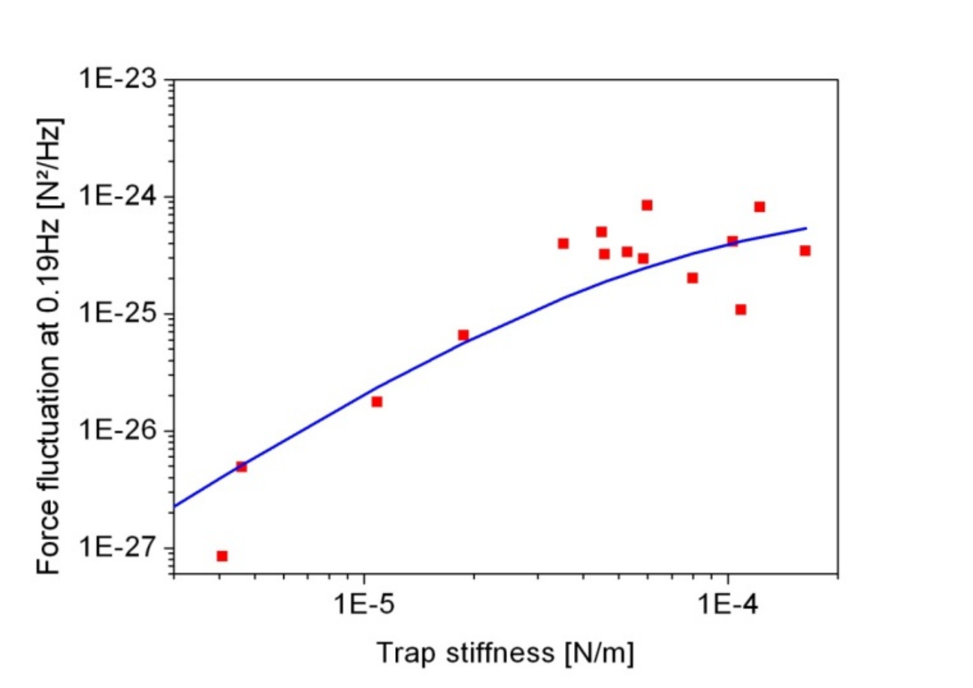



$$u_1 = -u_2 = -F/\left(k + 2k_{12}\right)$$

$$-k^2\left\langle u_1 u_2^*\right\rangle = \frac{k^2}{(k + 2k_{12})^2}\left\langle FF^*\right\rangle$$

# spring model

- internally generated forces deform cell and displace particles




- fitting $k_{12}$ and $<FF^*>$

$$-k^2\left\langle u_1 u_2^*\right\rangle = \frac{k^2}{(k+2k_{12})^2}\left\langle FF^*\right\rangle$$

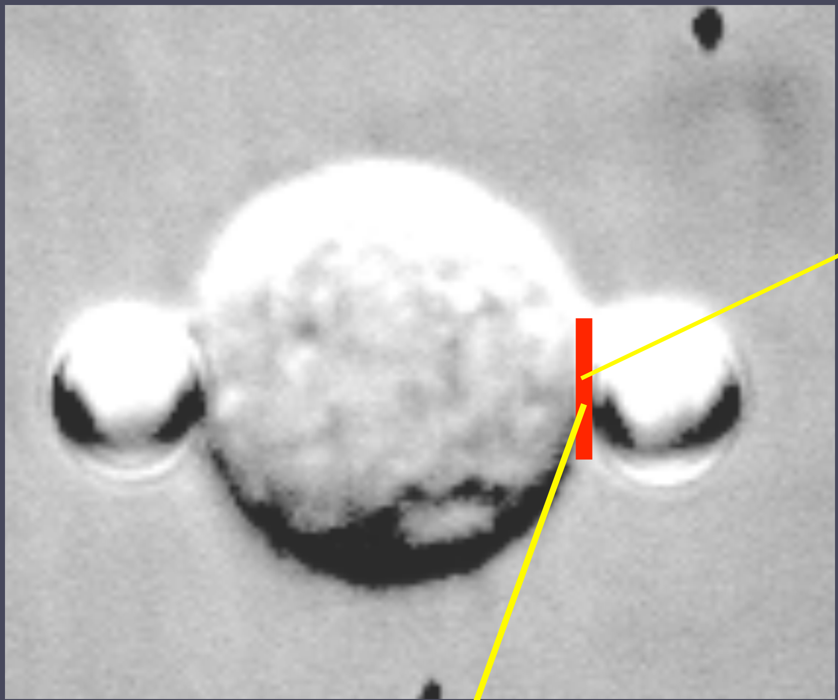
$$k_{Osteo} \approx 4\times 10^{-5}\, N/m$$

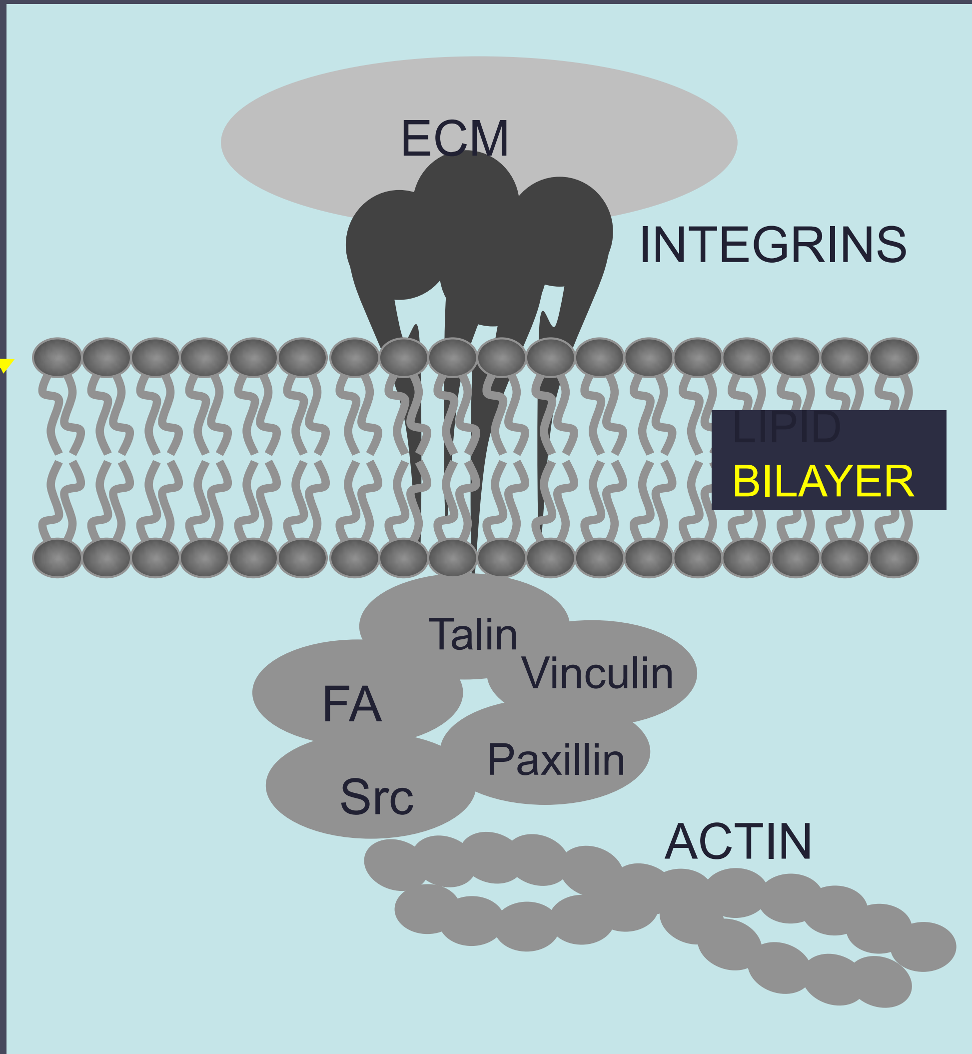
$$k_{Fibro} \approx 3\times 10^{-5}\, N/m$$

- results comparable to force-distance curve experiments

Mizuno et al. Phys Rev Lett (2009) 102: 168102

# mechanosensor : focal adhesion complex






total cellular force ??

"traction force" is applied to mechanosensor

# conclusions

— sampling of $\mu m^3$ volumes with microrheology (bandwidth 0.1 Hz- 100kHz)

— active MR more tedious, but better data

— active and passive MR ideal to study non-equilibrium soft systems

— myosin-motor driven actin networks show clear non-equilibrium

— $\omega^{-2}$ spectrum of non-equilibrium fluctuations, stiffening by internal stress

— force fluctuations of cells can be tracked, also show $\omega^{-2}$ spectrum

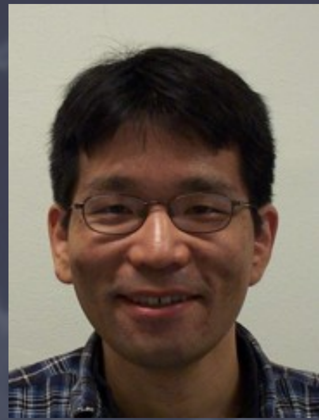
— van Hove correlation function distinguishes motors: far away/close by.

acknowledgements ->

# the people involved:

## Georg-August-Universität Göttingen

*Physics*
Lena Lautscham
Florian Schlosser
Florian Rehfeldt
Daisuke Mizuno



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Toshihiro Toyota
Daisuke Mizuno

*Funding:*
FOM (Netherlands)
DFG SFB 755 (Germany)
DFG CMPB (Germany)
KAKENHI (Japan)