Mechanosensation of Adherent Cells

MBCM, Banff, Aug 2011

JOHNS HOPKINS
UNIVERSITY

Acknowledgements

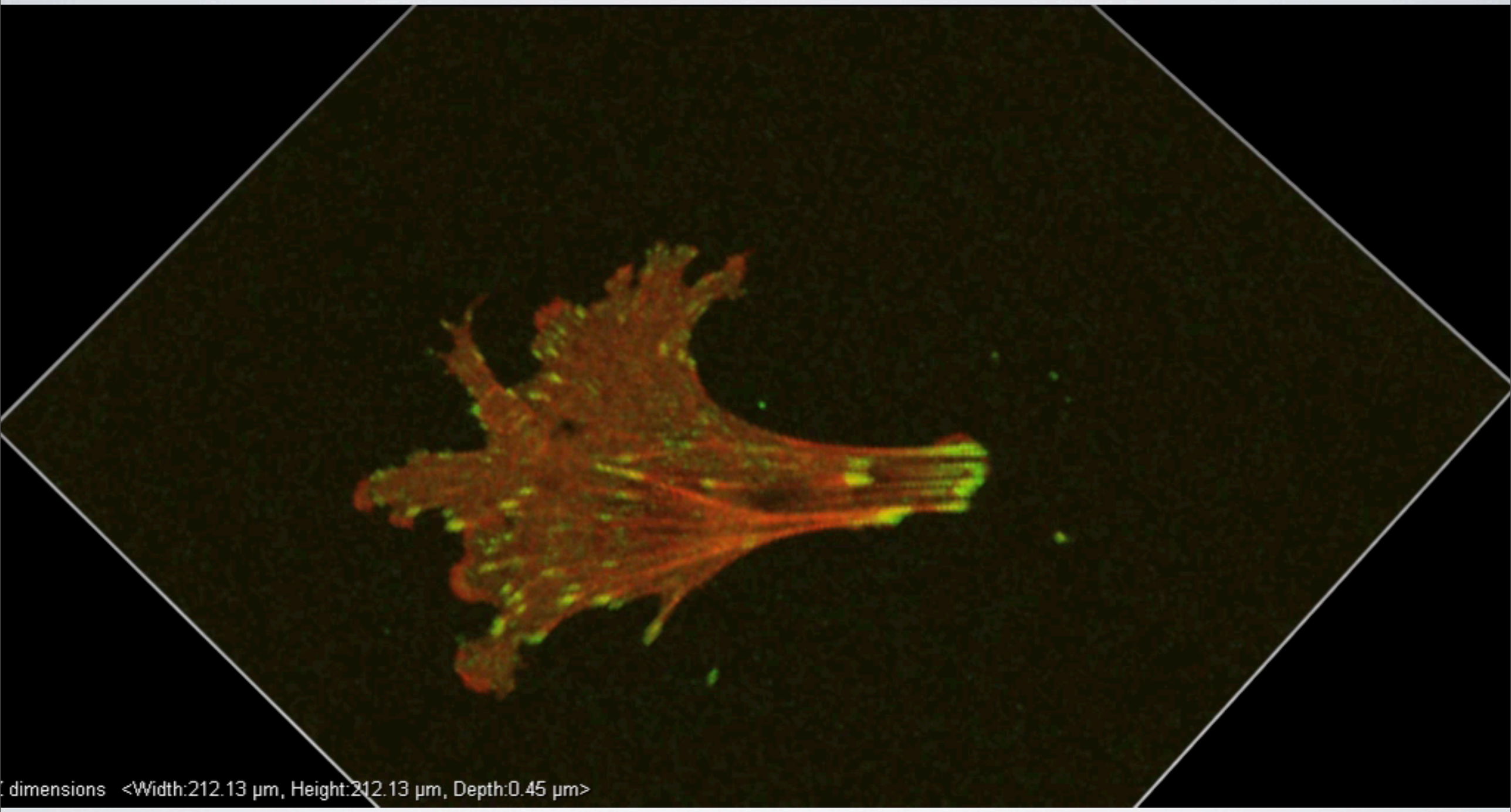
- **Sam Walcott**
- **Hongyuan Jiang**
- **Ben Harland**
- **Alex Dajkovic**
- **Jin Seob Kim**
- **Alfredo Celedon**
- **Osman Yogurtcu**
- **Ganhui Lan**
- **Fangwei Si**

Denis Wirtz (JHU)

NSF, NIH

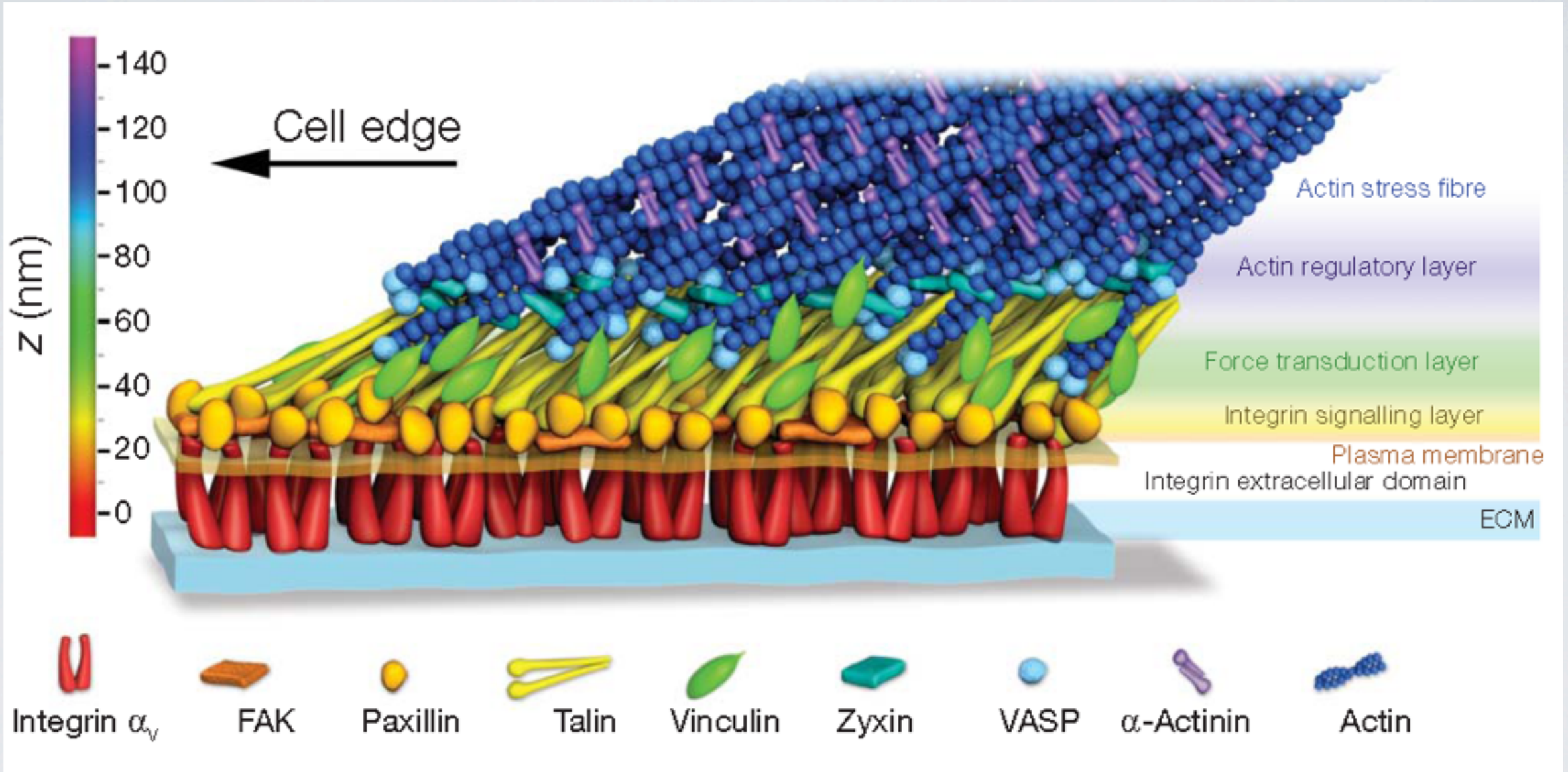
JOHNS HOPKINS
UNIVERSITY

Cells plated on a substrate



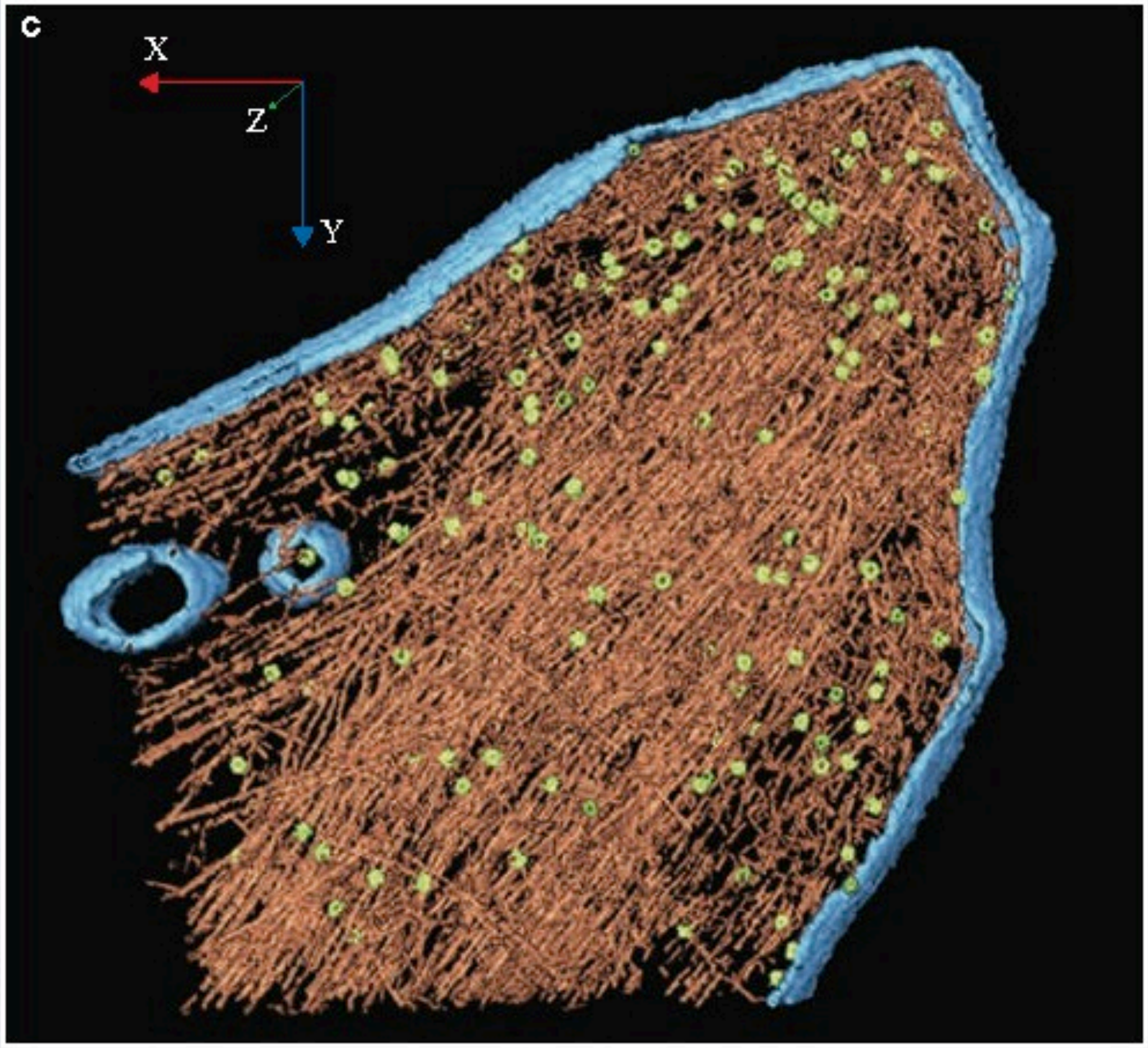
Courtesy of lab of Denis Wirtz

What does a focal complex look like?



Kanchanawong. *Nature*. (2010).

Still unclear on the ultra-structure



Palta et al. *Nature Cell Biol*. (2010).

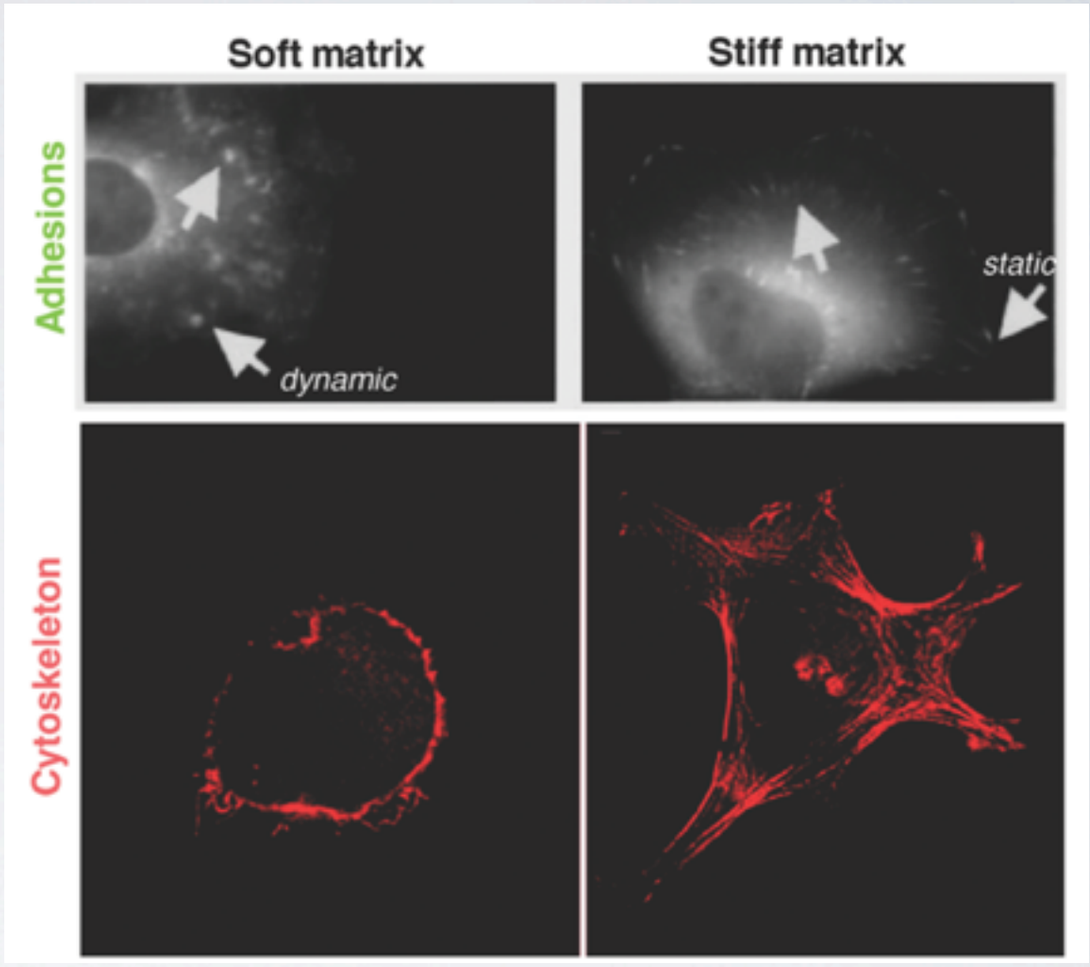
Mechanosensation of cells

For a review: Discher Janmey and Wang, Science. 310, 1139-1143 (2005).

Stem cells must be plated on a 2D surface to survive *in vitro.*

Depending on the stiffness of this surface, cells differentiate into different lineages (soft = neuron; intermediate = Muscle; stiff = Bone).

The cell cytoskeleton and surface adhesion structures also depend on surface stiffness.



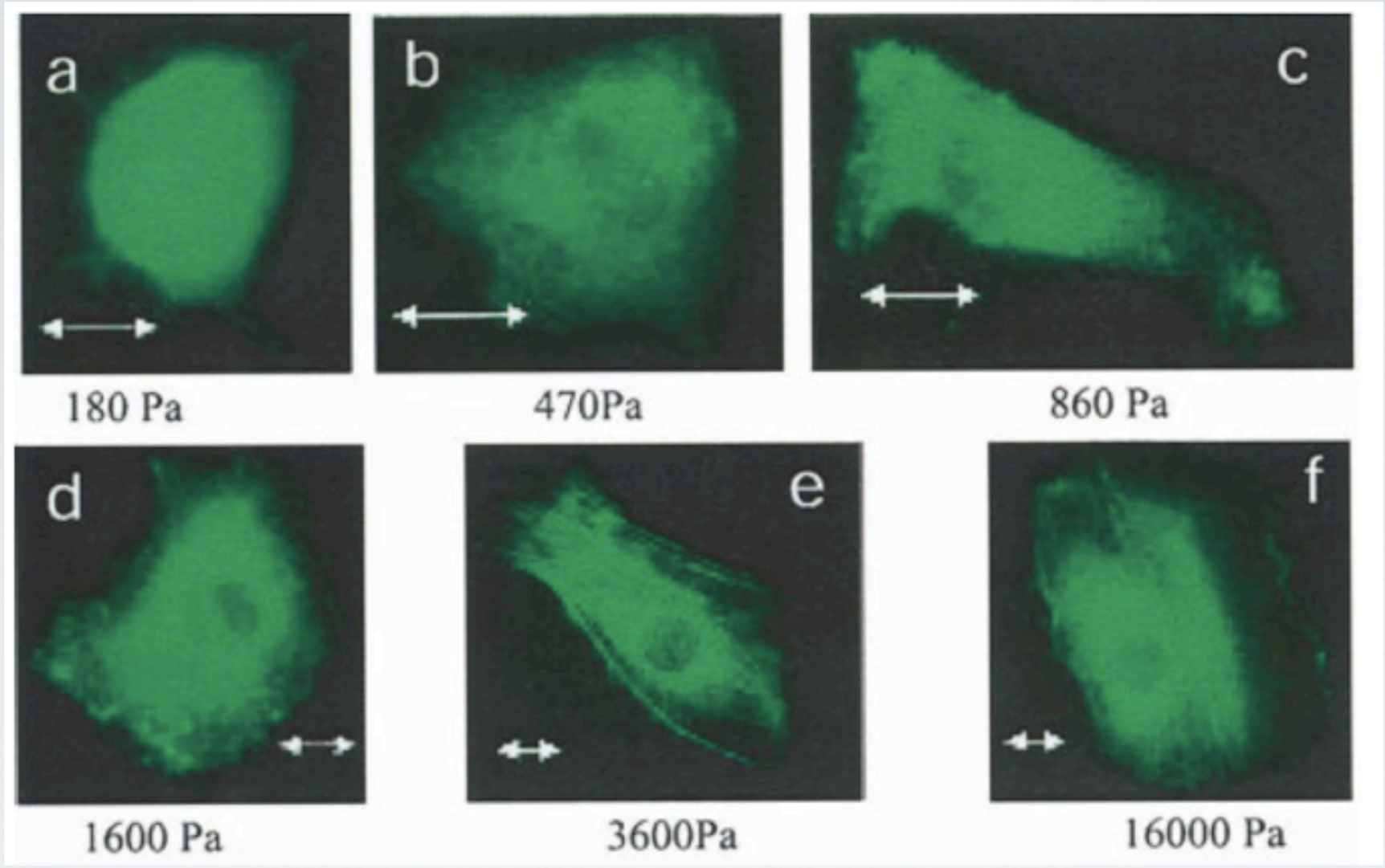
Most are thinking about the biochemical mechanism that rise to this phenomena.

Can we understand mechanosensation through mechanics?

Is mechanics the origin of biochemical responses?

JOHNS HOPKINS
UNIVERSITY

Cytoskeleton



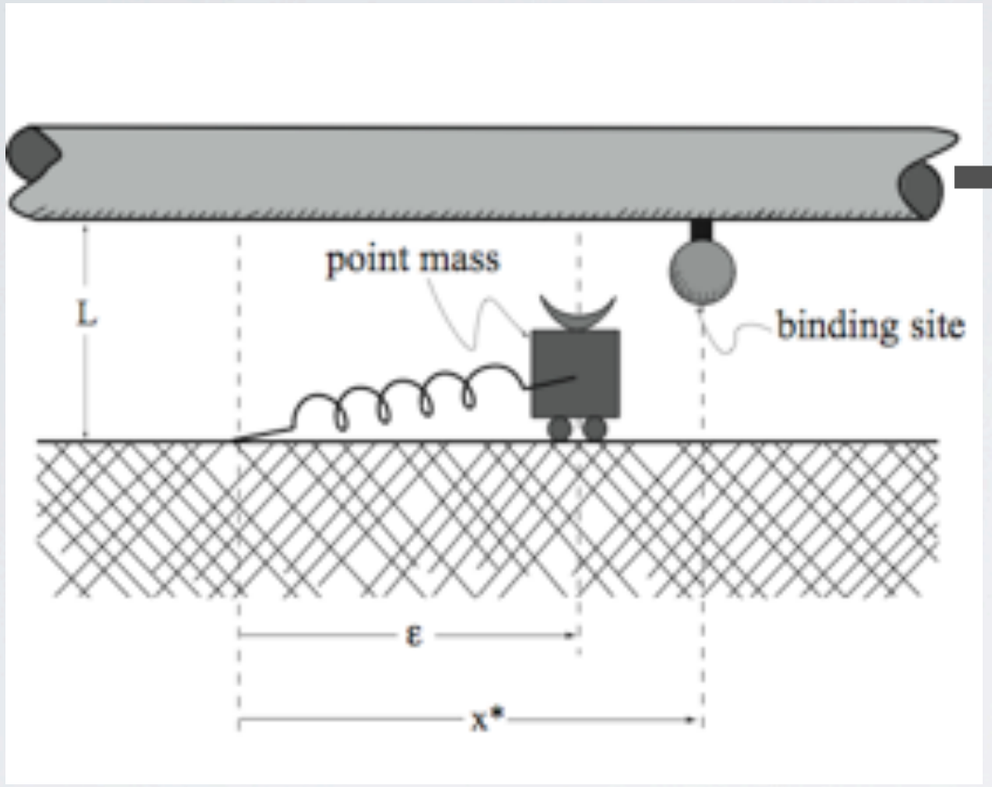
From Yeung et al., *Cell Mot. Cyt.* **60**, 24-34 (2005).

As surface stiffness increases, the actin cytoskeleton goes from being diffuse to being arranged into "stress fibers."

Actin cross-linking proteins (a-actinin & fascin) are associated with bundles.

JOHNS HOPKINS
UNIVERSITY

Forces in regulating chemical reactions



Applied force, F

From thermal fluctuations there is a probability to reach the binding site, characterized by a rate constant.

$$k_a \approx k_0 \exp\left(\frac{Fx_c - F^2\frac{1}{2}\left(\frac{1}{\kappa_t}+\frac{1}{\kappa}\right)}{k_b T}\right)$$

approx.

$$k_a \approx k_0 \exp\left(\frac{Fx_c}{k_b T}\right)$$

Bell's model

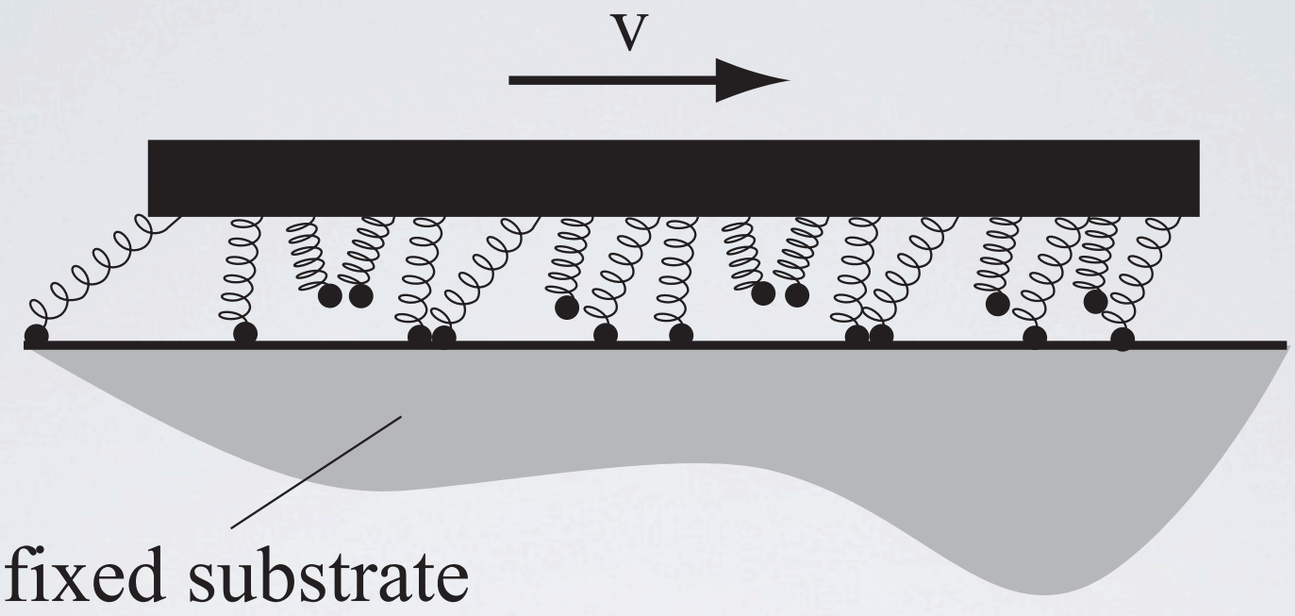
G.I. Bell. *Science*. (1978).

Attachment rate

Change in the transition state energy

JOHNS HOPKINS UNIVERSITY

What about an ensemble of binding sites?



What is the probability density of finding a spring bound with strain x at time t? (Lacker, 1997; Lacker & Peskin, 1986).

Prob. density of being bound with strain x at time t

Strain-dependent attachment rate function prob. density

$$\frac{\partial n}{\partial t} + v\frac{\partial n}{\partial x} = -g(x)n + f(x)\left(1 - \int_{-\infty}^{\infty} n dx\right)$$

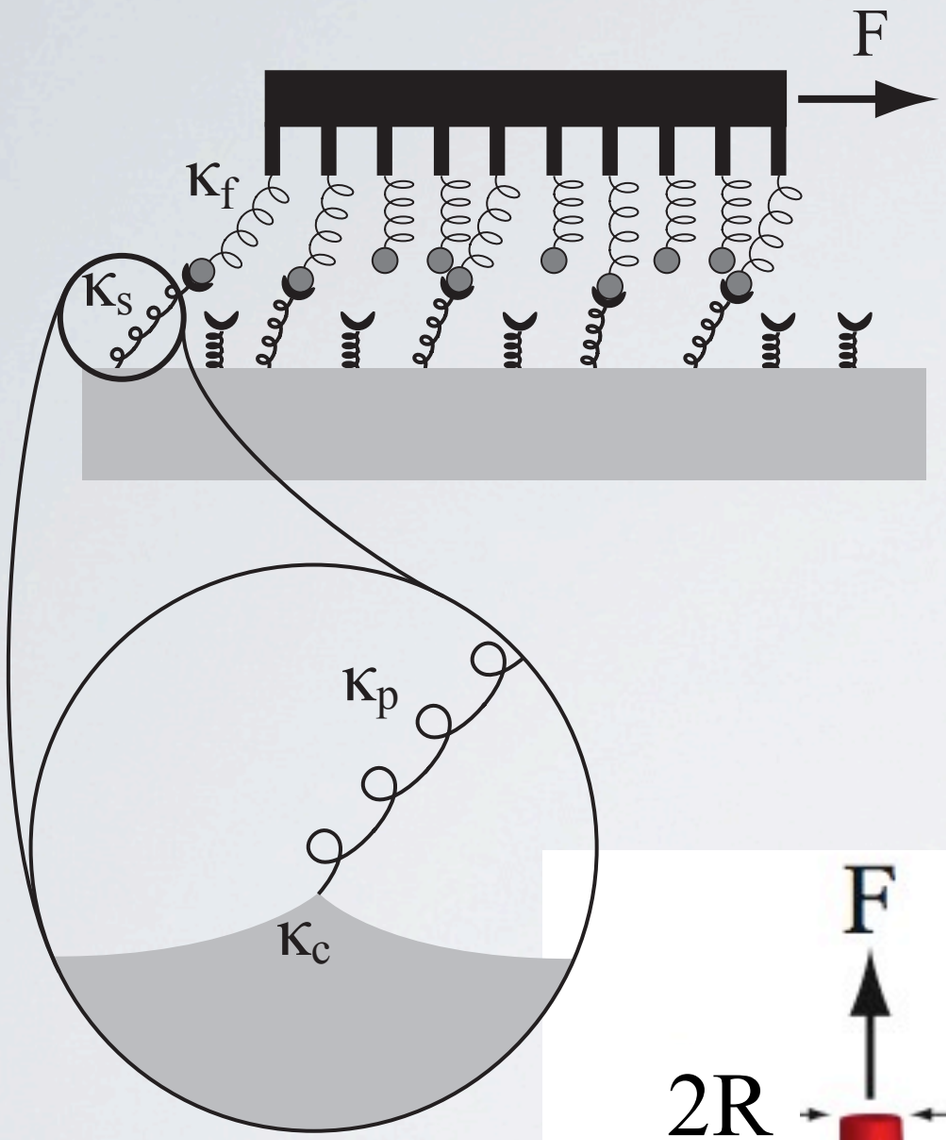
Strain-dependent detachment rate function

Strain

$$F = \int n(x,t)[\text{force} - \text{strain}]dx \qquad F = \int n(x,t)\,[\kappa x]\,dx$$

JOHNS HOPKINS
UNIVERSITY

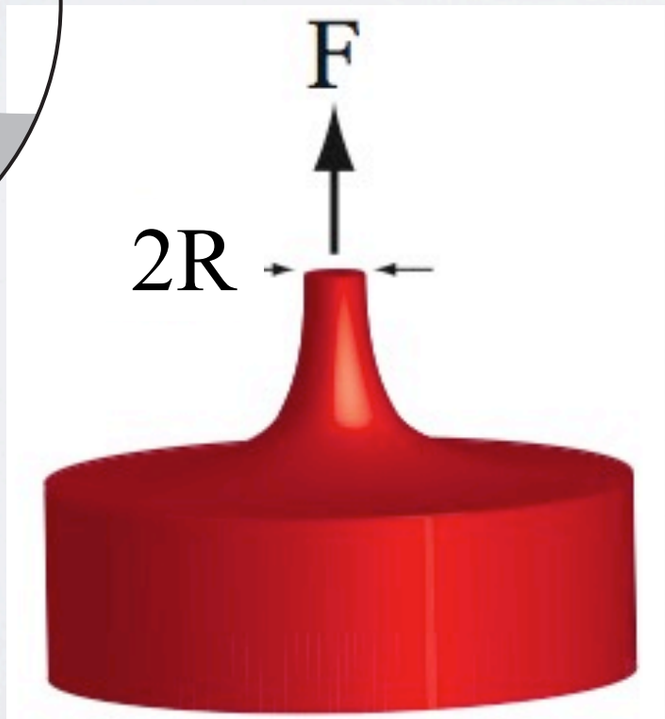
Stiffness of the substrate



Binding sites on the surface (with stiffness κ_s) interact with proteins anchored in the focal contact (with stiffness κ_f).

Binding site stiffness is made up of two terms: the stiffness of the surface κ_c and the stiffness of the proteins κ_p.

$$\frac{1}{\kappa_s} = \frac{1}{\kappa_c} + \frac{1}{\kappa_p}$$



Assuming that the surface is linear elastic, homogenous and isotropic, and that a constant force is applied over a disc of radius R, we can write an effective spring constant for the surface:

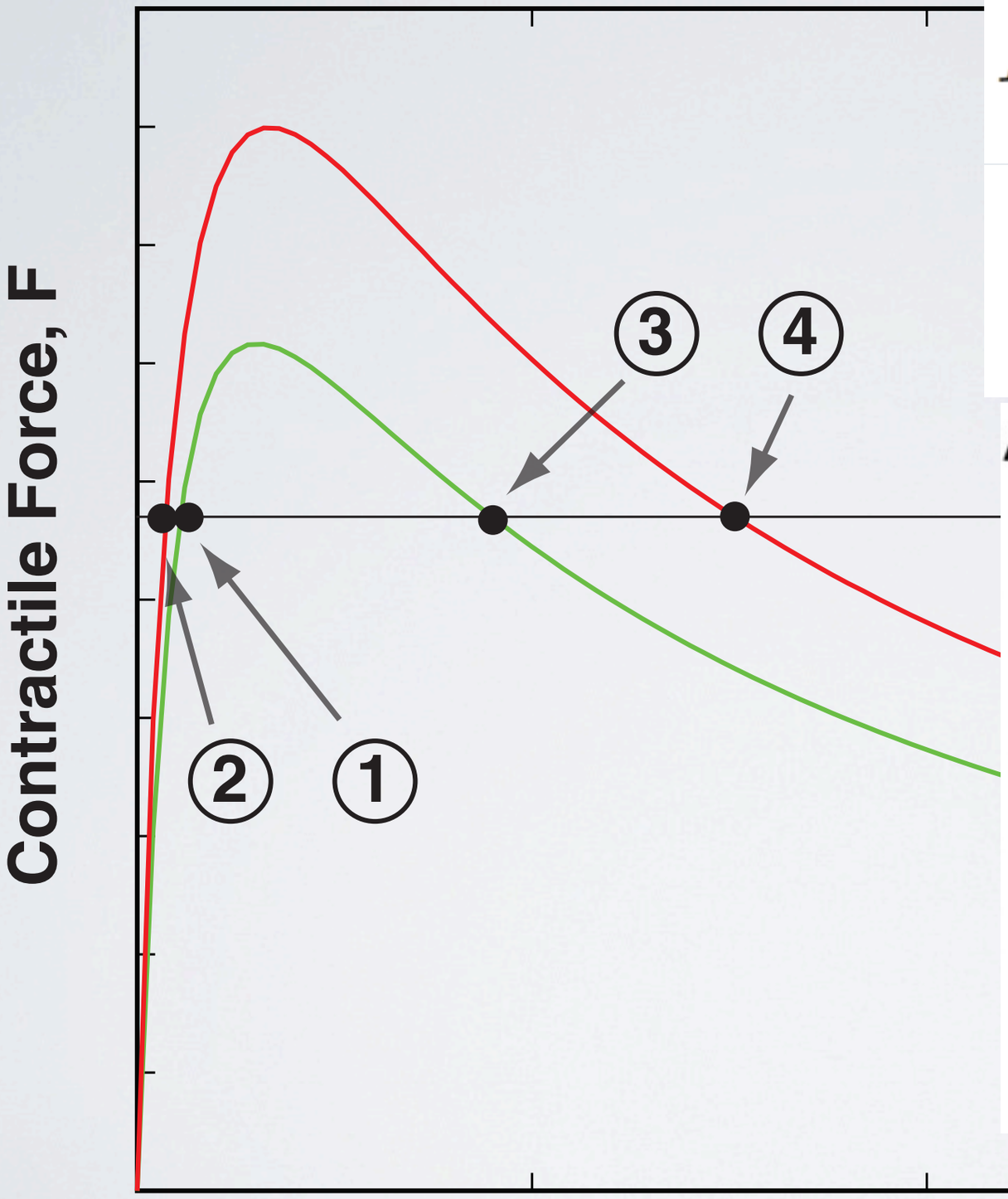
$$\kappa_c = \frac{\pi R E}{2(1+\nu)(1-\nu)}$$

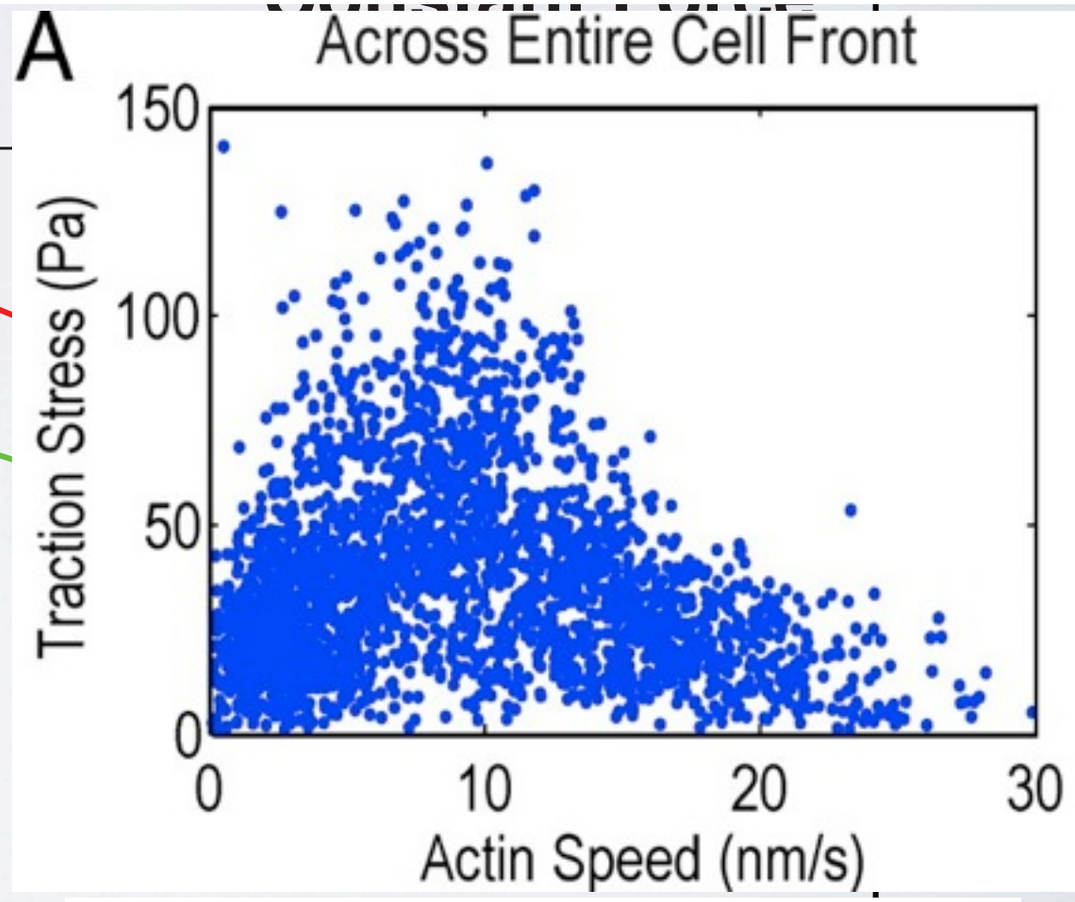
JOHNS HOPKINS
UNIVERSITY

Steady state F-V shows rich phenomena

$$F \approx M\bar{\kappa}\frac{f_1}{g_0}\frac{1}{f_1+g_0}v = bv$$

$$\bar{\kappa} = \kappa_{eff}\frac{2\pi RE}{2\pi RE + 3\kappa_{eff}}$$

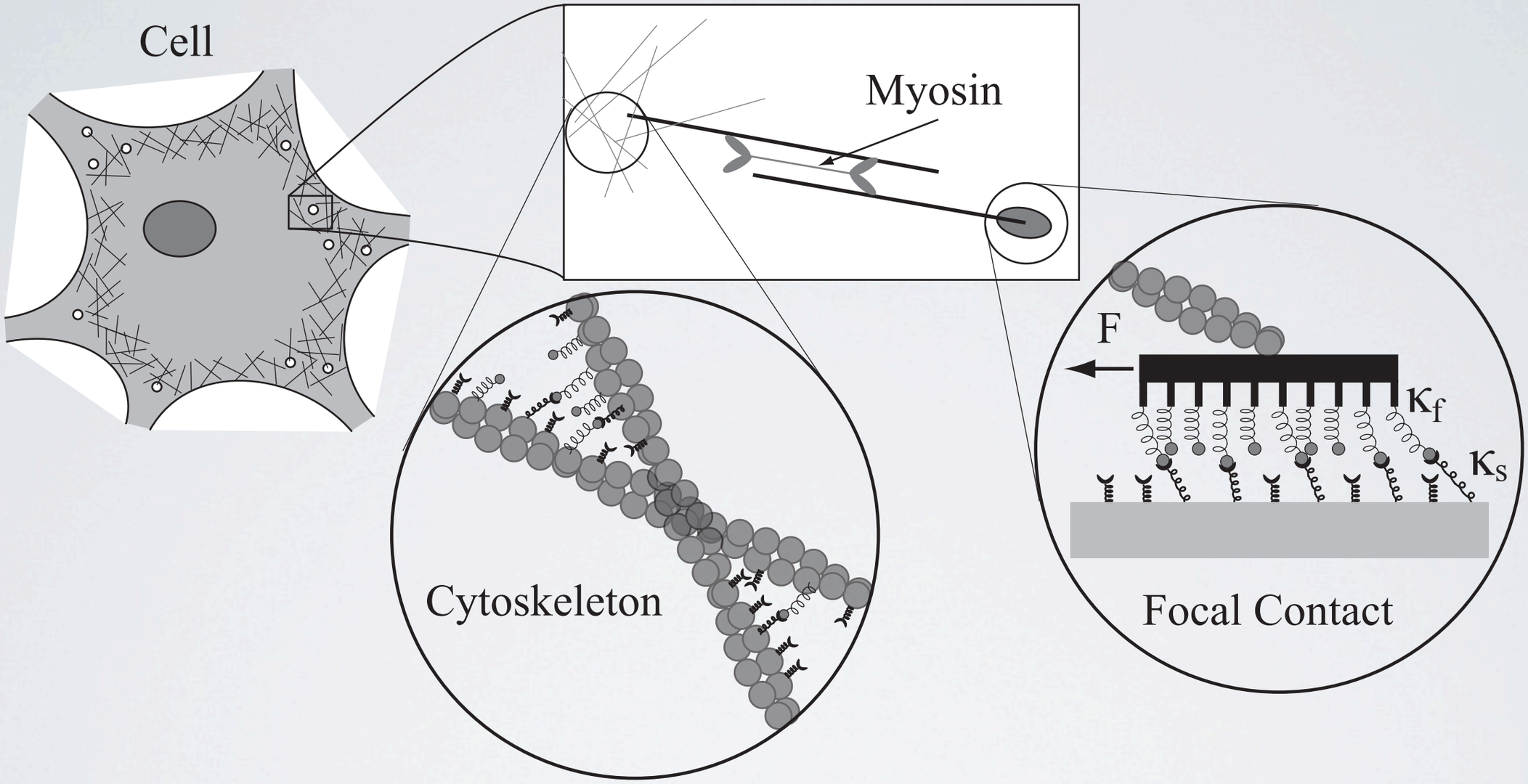




Gardel et al, J. Cell Biol. 2008

JOHNS HOPKINS UNIVERSITY

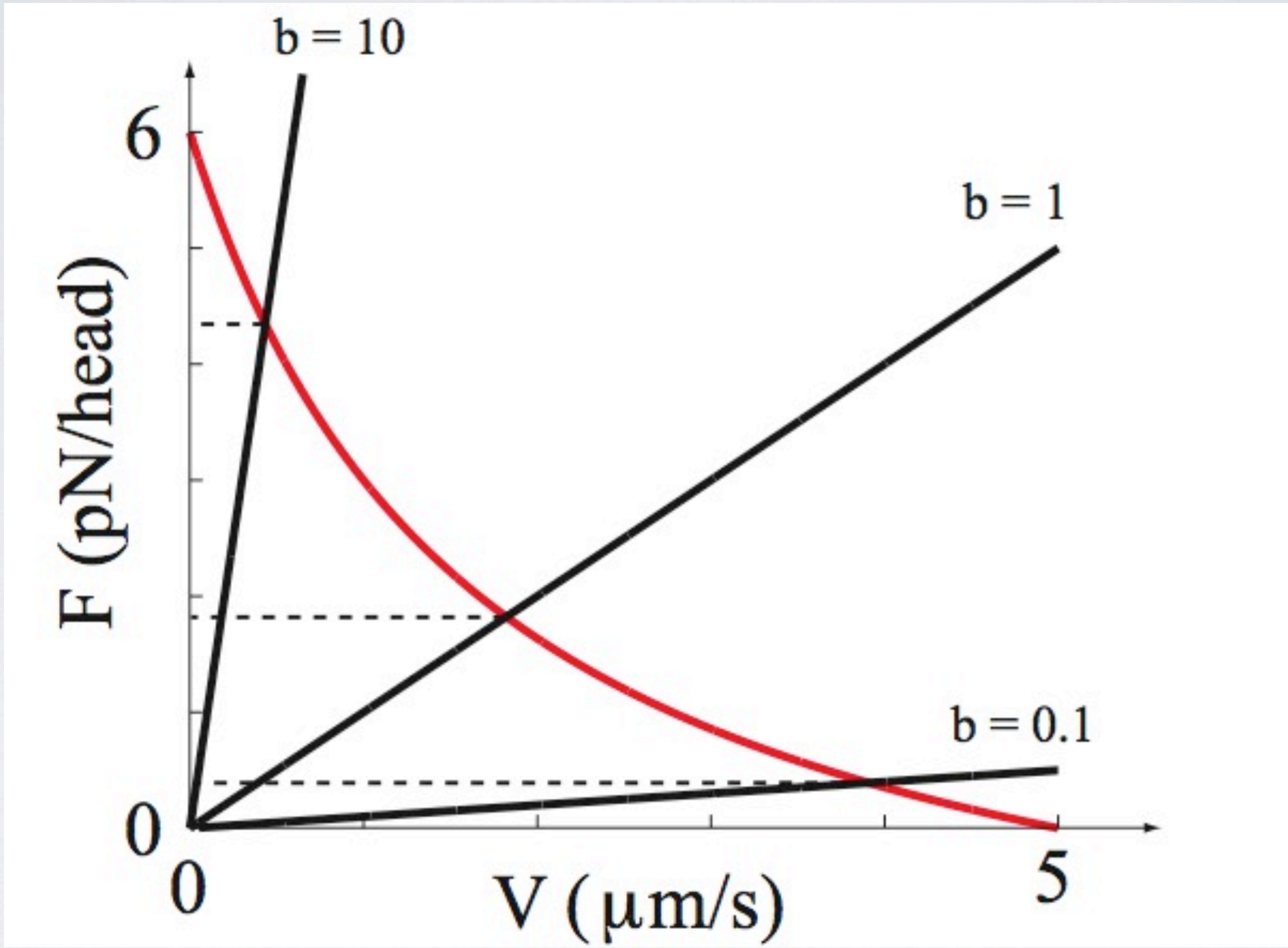
A Physics-based Model



1. Friction model of focal contacts. 2. Myosin model. 3. Friction model of cytoskeleton.

JOHNS HOPKINS
UNIVERSITY

Myosin force balances adhesion friction

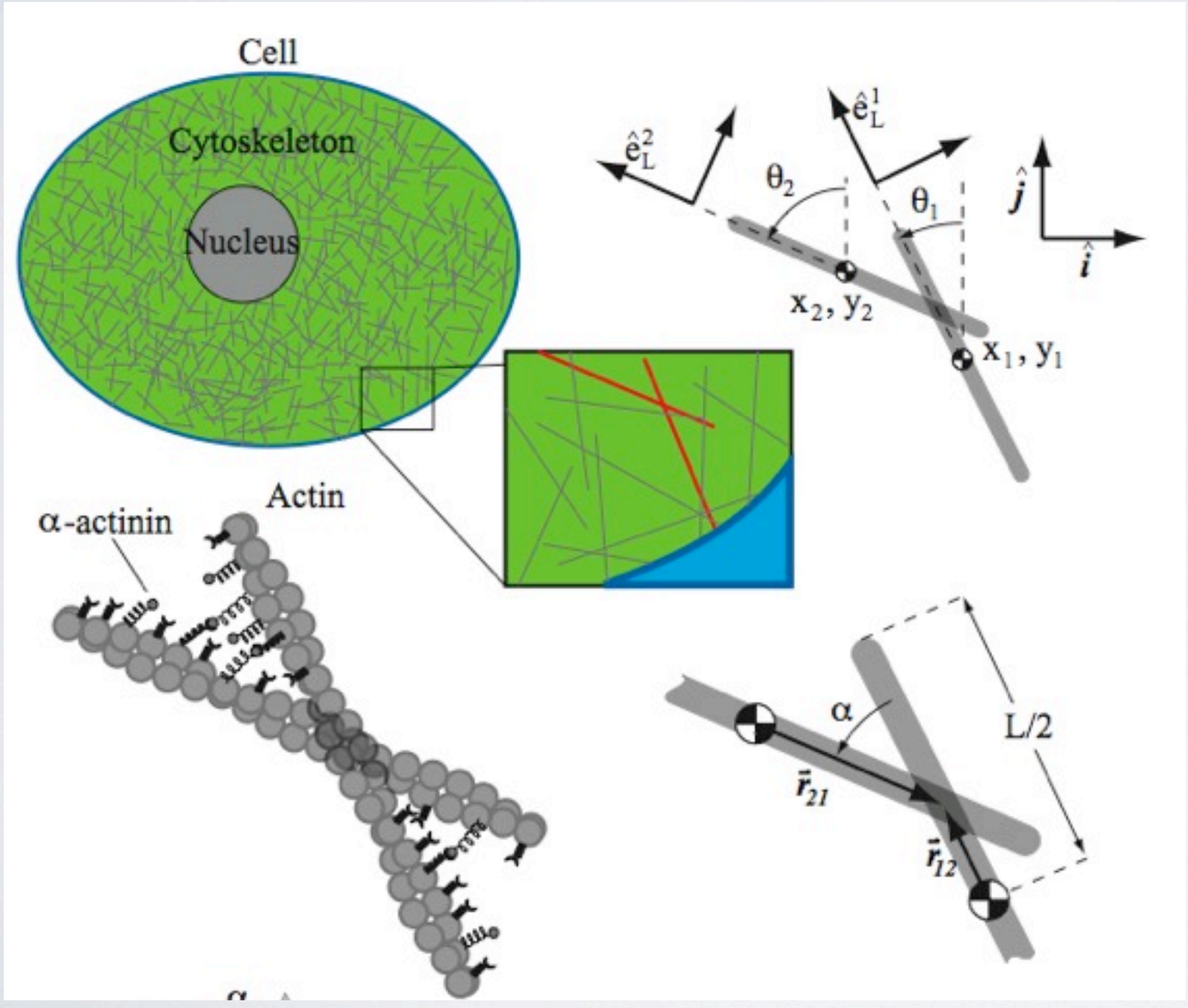


JOHNS HOPKINS
UNIVERSITY

Cytoskeleton: friction between actin filaments

The cytoskeleton is (at least initially) a random arrangement of actin filaments.

Actin-binding proteins, **a-actinin, fascin, zxyin**, cause friction between the filaments.



Using our model for friction:

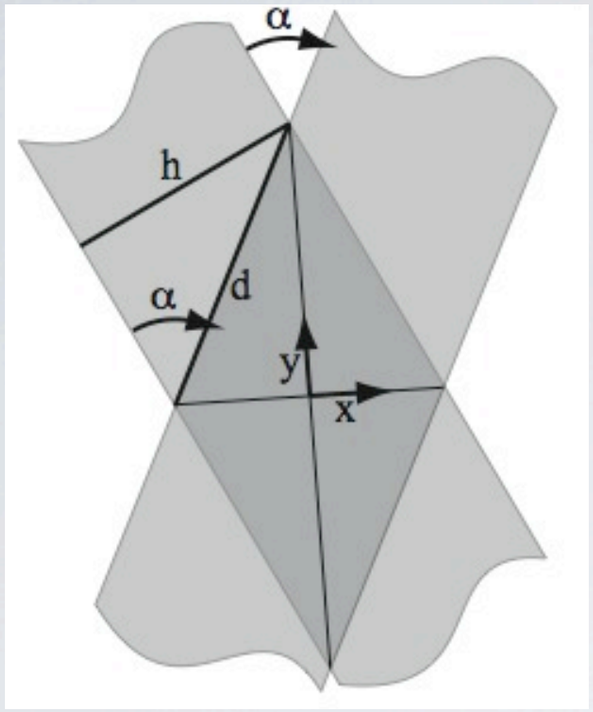
$$F \approx M\bar{\kappa}\frac{f_1}{g_0}\frac{1}{f_1+g_0}v = bv$$

the drag constant depends on the relative angle between filaments (because the area of contact is angle dependent).

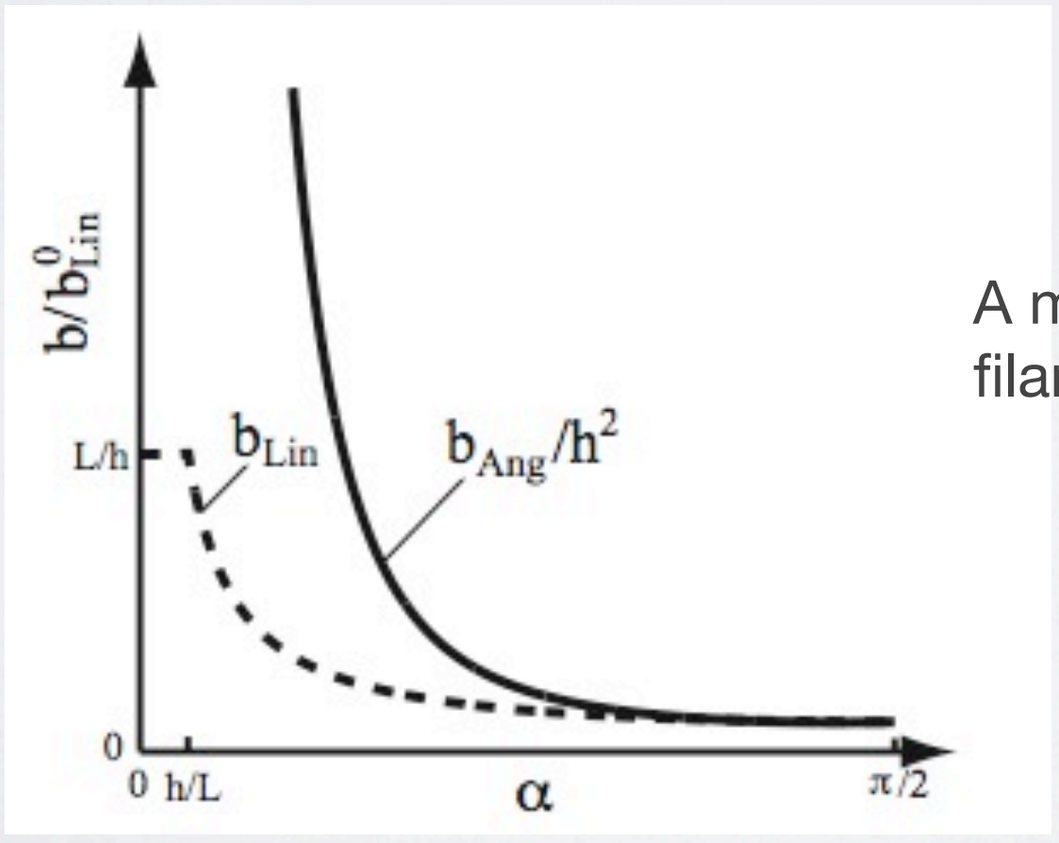
There is also torsional drag.

JOHNS HOPKINS
UNIVERSITY

Angular friction depends on filament overlap



The more parallel the filaments (smaller the relative angle), the greater the drag.



A maximum is reached when the filaments fully overlap.

JOHNS HOPKINS
UNIVERSITY

Equations for an ensemble of interacting filaments

$$\sum_j \mathbf{F}_{ij} + F^i_{\text{ext}} = 0$$

$$\sum_j T_{ij} = 0$$

Non-dimensionalize

$$\sum_j \bar{\mathbf{F}}_{ij} + \sum_k \delta_{ik} \mathbf{e}^i_f$$

$$\sum_j \bar{T}_{ij} = 0$$

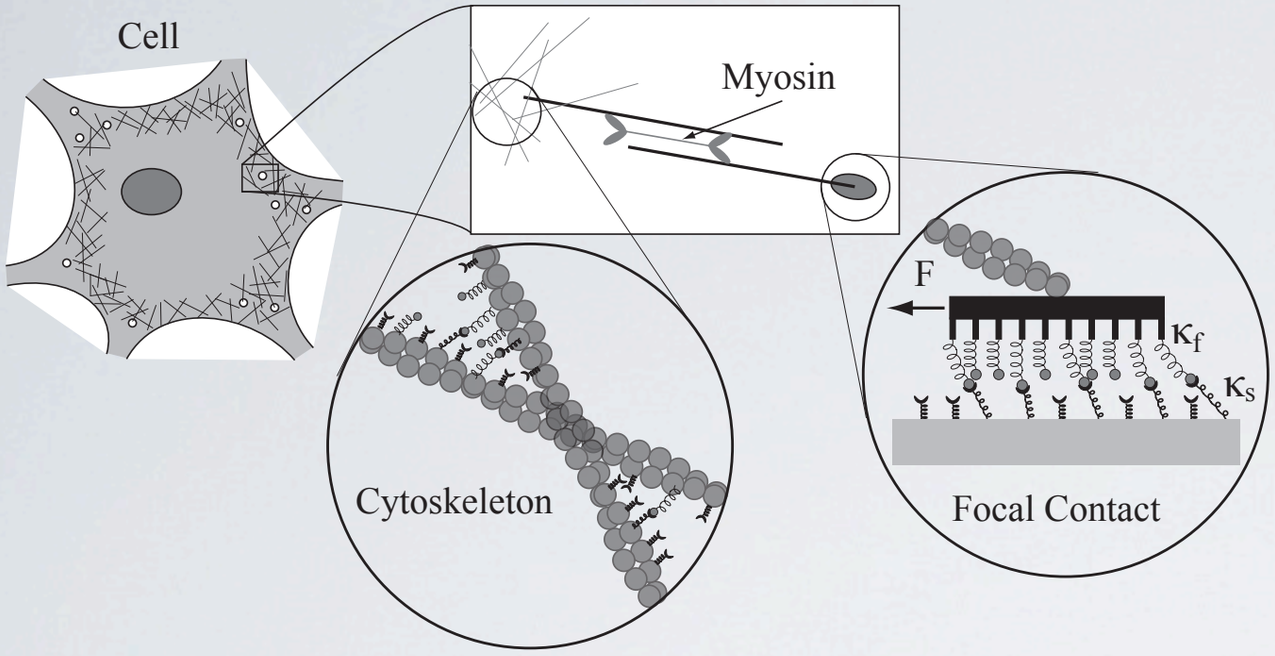
Relative frictional force, depend on overlap

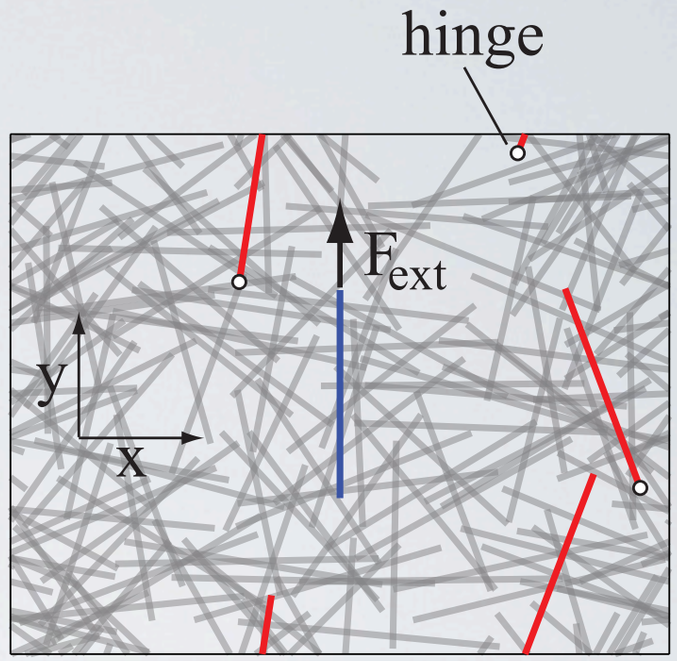
Relative angular friction

1 free parameter remaining: aspect ratio of filaments.

JOHNS HOPKINS
UNIVERSITY

Emergence of a time scale





Myosin applies a load between focal contacts and the cytoskeleton.

We simulate this situation by applying a constant load to a small number of filaments.

A few fixed filaments mimic cytoskeleton-surface interactions.

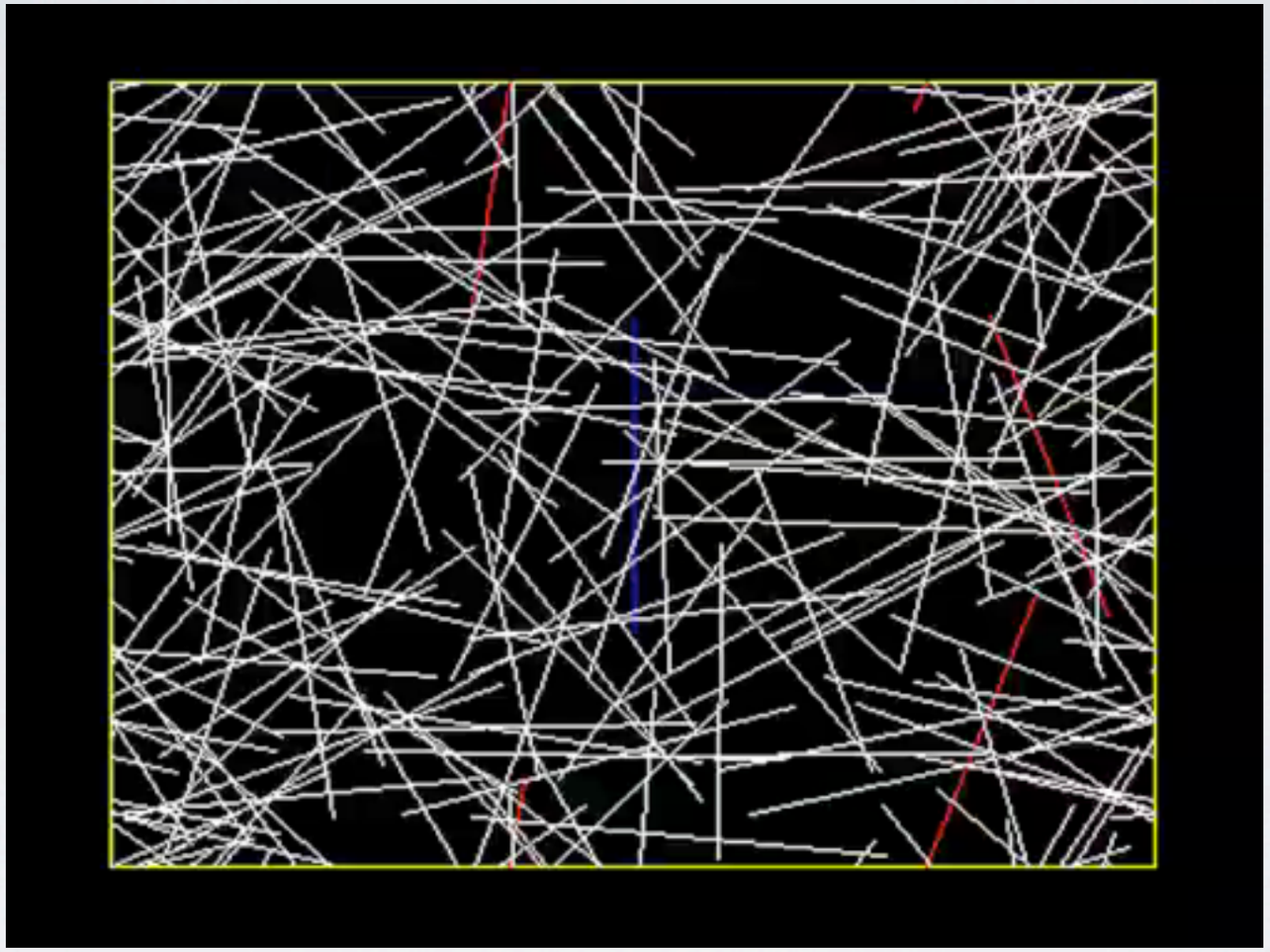
Given an aspect ratio of the filaments, there are no free parameters.

Varying the applied force, filament length and/or the drag between filaments simply changes the time scale:

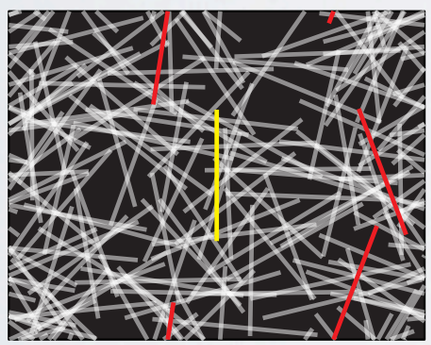
$$\tau = \frac{b_{act} L}{F_{ext}}$$

JOHNS HOPKINS
UNIVERSITY

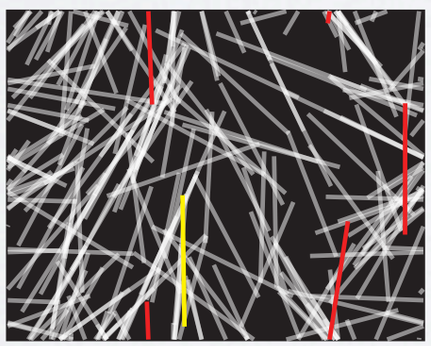
Simulation results



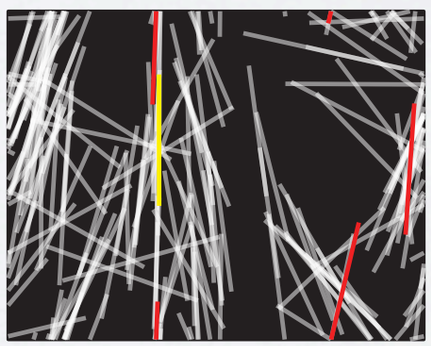
$\tau = 0$



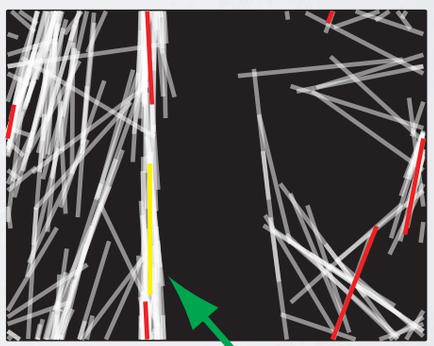
$\tau = 150$



$\tau = 300$



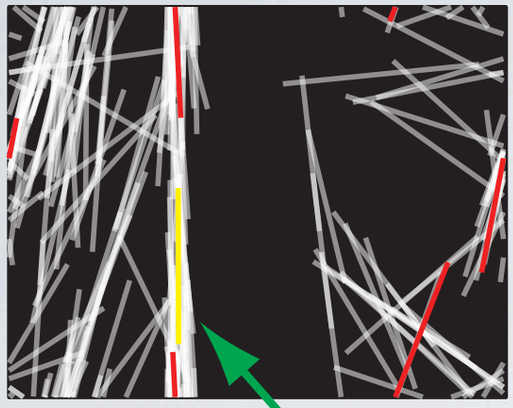
$\tau = 450$



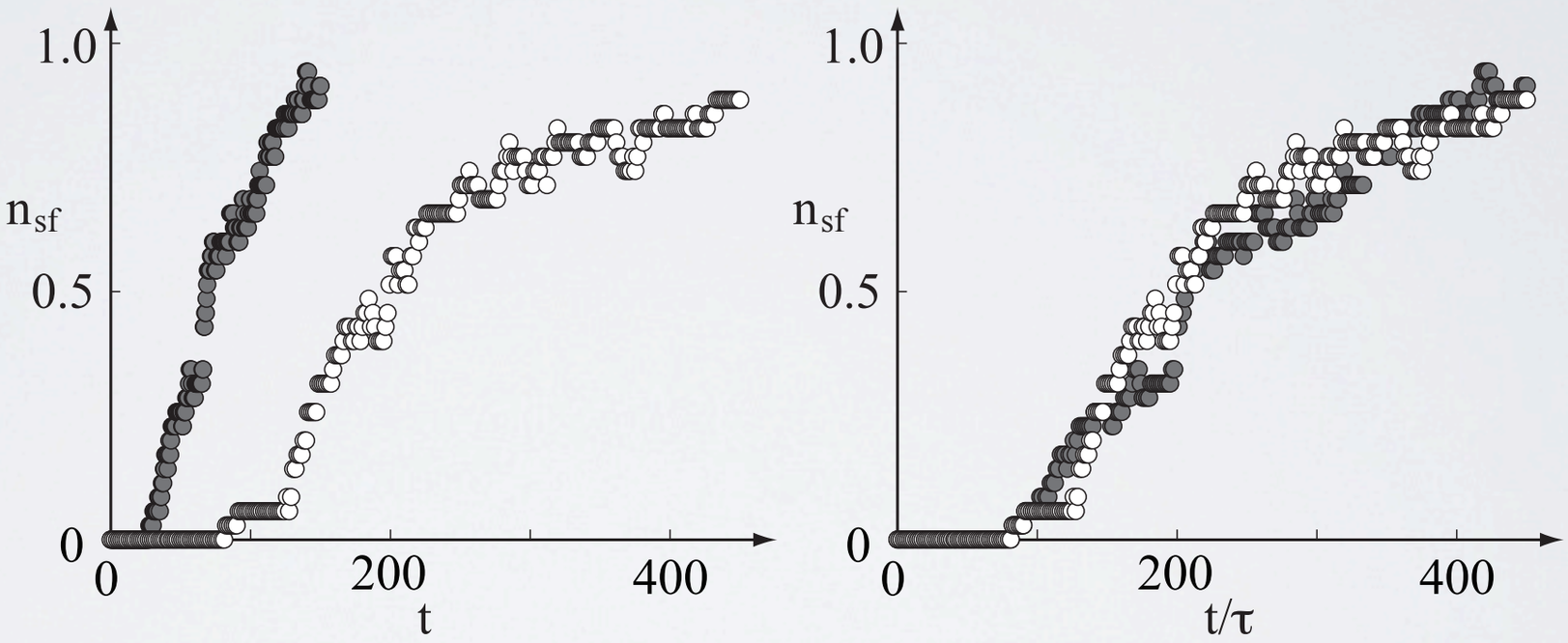
Stress fiber

JOHNS HOPKINS
UNIVERSITY

Simulation results cont.



Define a “stress fiber” of size N as being N or more filaments whose orientations are within +/-θ_c of the applied force direction AND whose centers of mass are all within +/-x_c



Dark points: b_{act}=10, F=30, L=1 $\tau=b_{act}L/F=1/3$

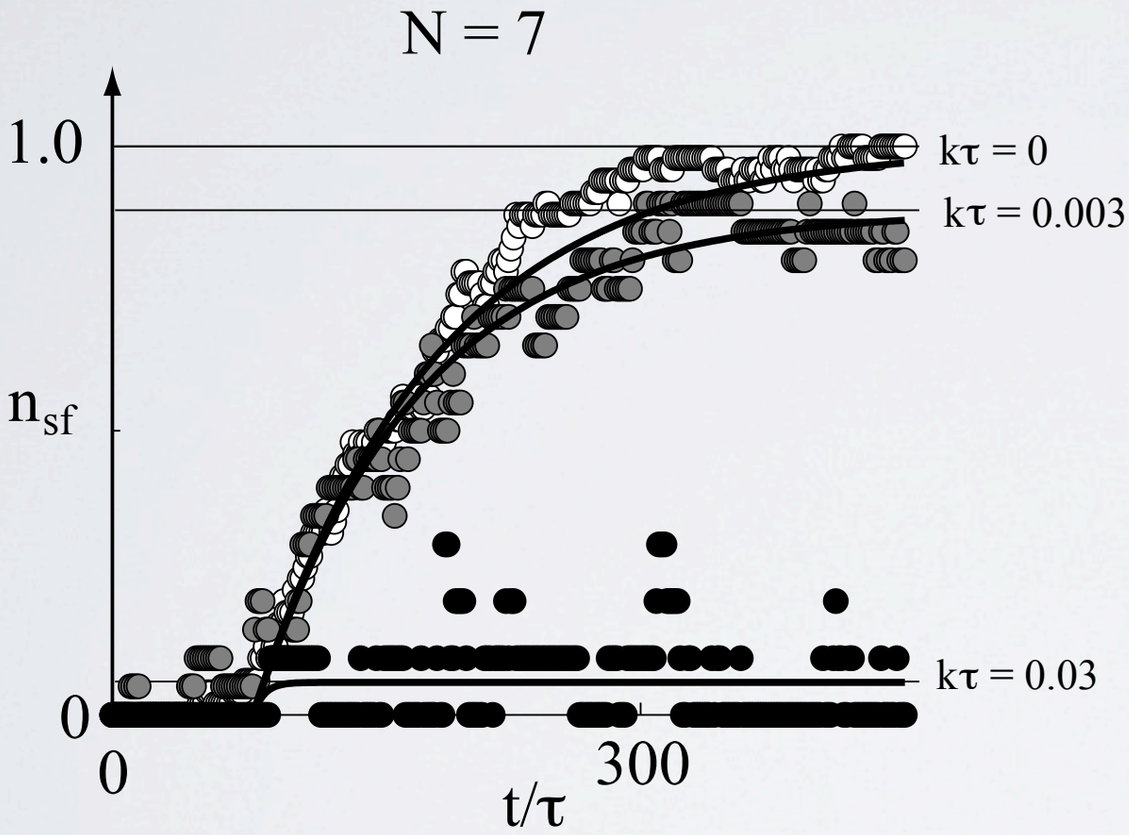
Light points: b_{act}=100, F=100, L=1 $\tau=b_{act}L/F=1$

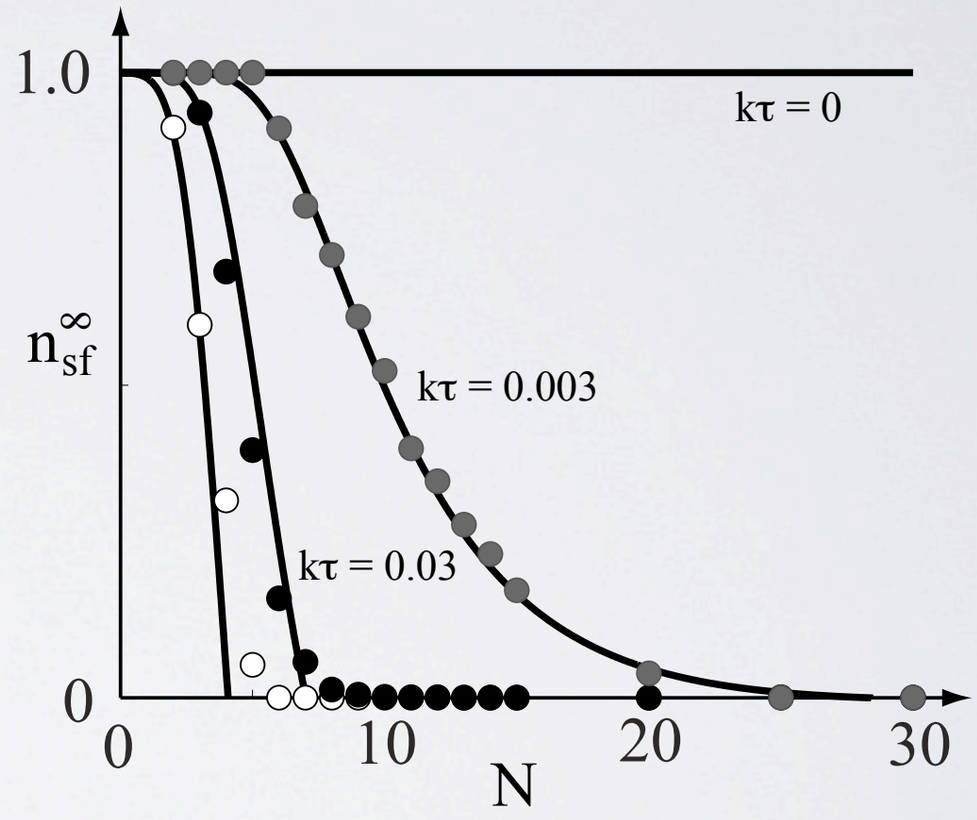
JOHNS HOPKINS
UNIVERSITY

Timescale II: actin turnover

In these simulations, given sufficient time, all (or at least most) filaments form one large bundle.

However, in a cell, the actin cytoskeleton is not static. It is constantly being broken down and replaced. What happens when we introduce this second time scale?





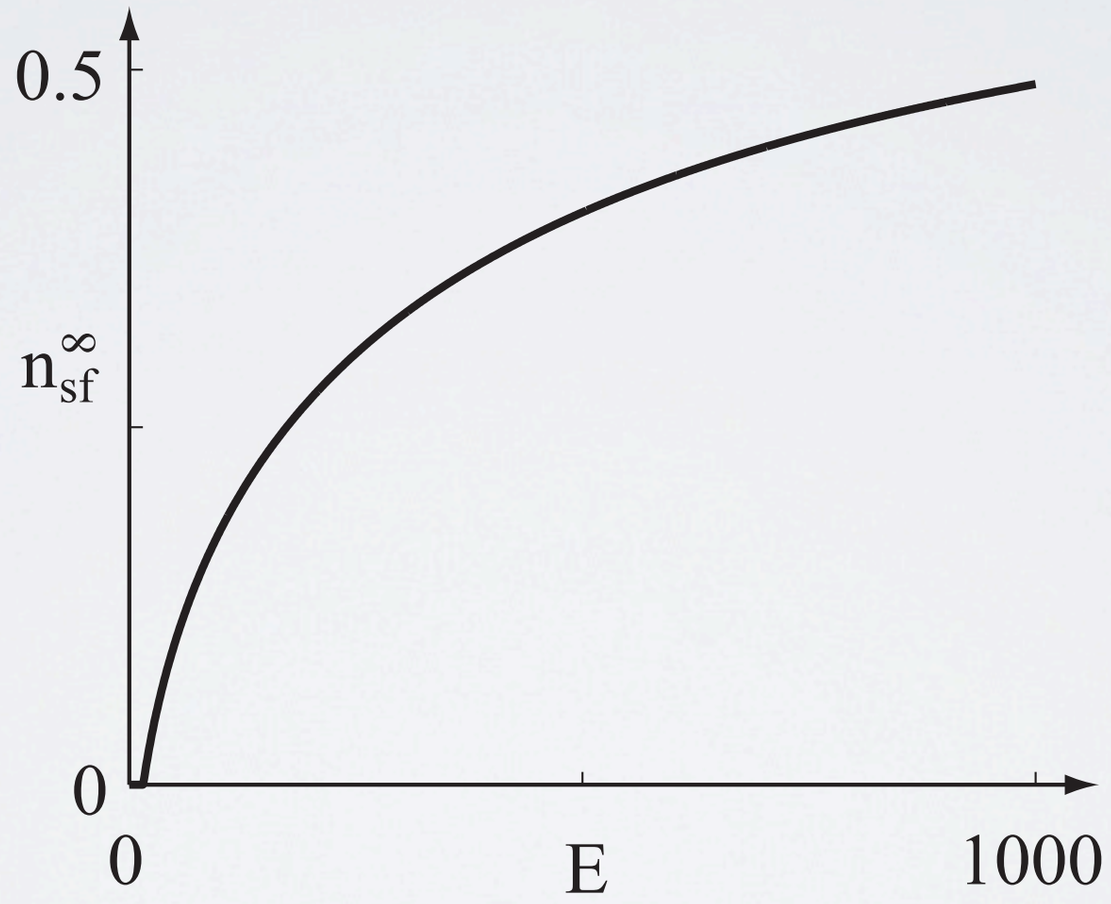
Thus, including actin turnover, the steady-state probability of forming a stress-fiber depends on the cytoskeleton time scale ($\tau = b_{act}L/|F|$).

JOHNS HOPKINS
UNIVERSITY

Take home message:

The steady-state number of stress fibers is surface stiffness dependent.

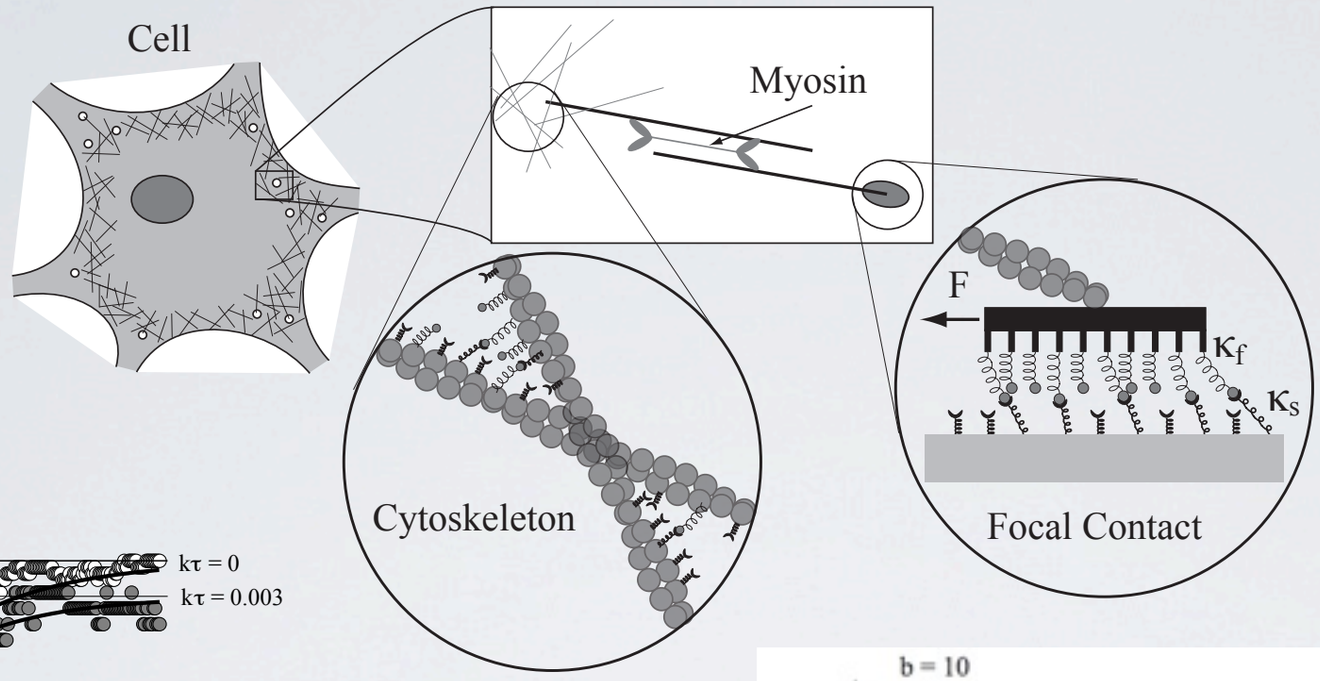
This model explains the difference in cytoskeleton organization and focal contact “stability” as a function of surface stiffness.

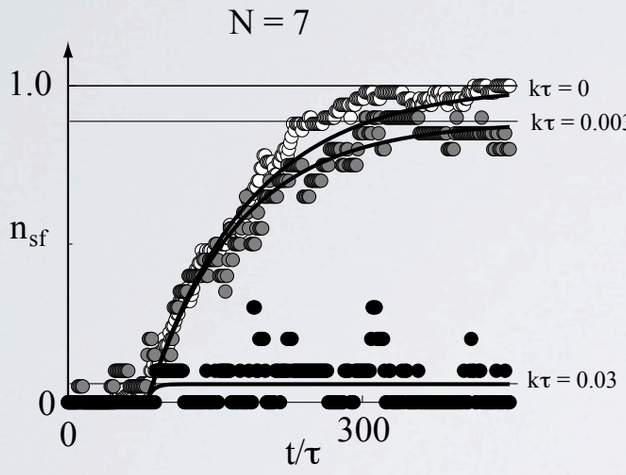


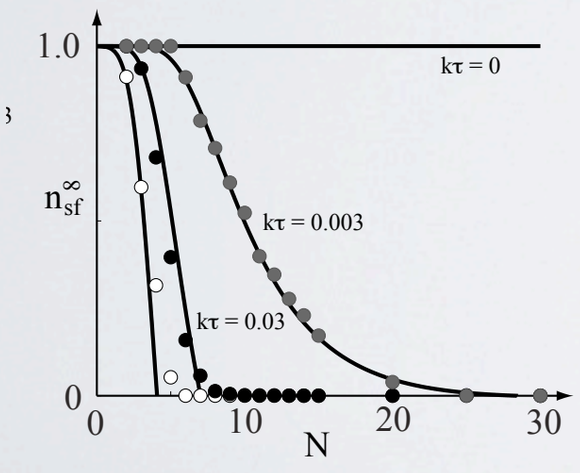
N=25, $k_{to}=10^{-6}s^{-1}$, L=1μm, c=4, v_0=50nm/s, F_0=100pN, r=200nm, κ_{eff}=1pN/nm, b_{act}=100pN s/μm

JOHNS HOPKINS
UNIVERSITY

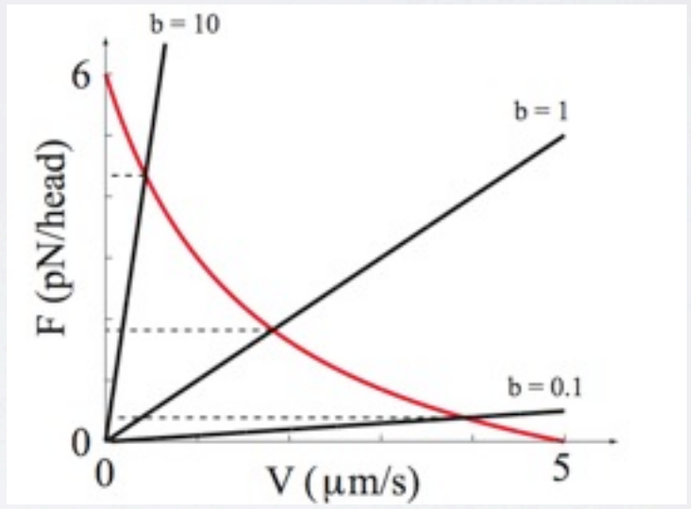
Putting it all together



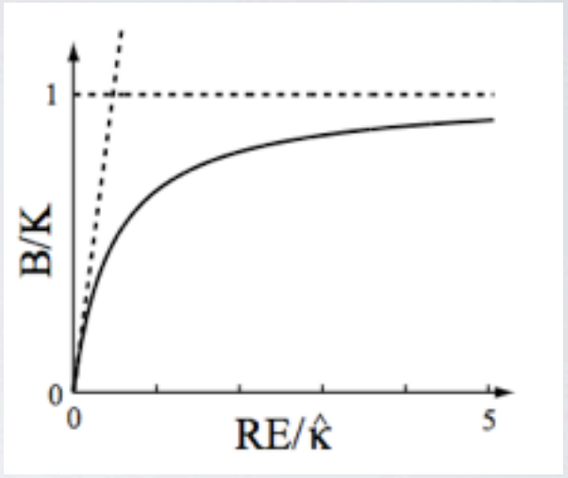




$$\tau = b_{act} L / |F|$$



Adhesion complexes: sense surface stiffness through drag coefficient

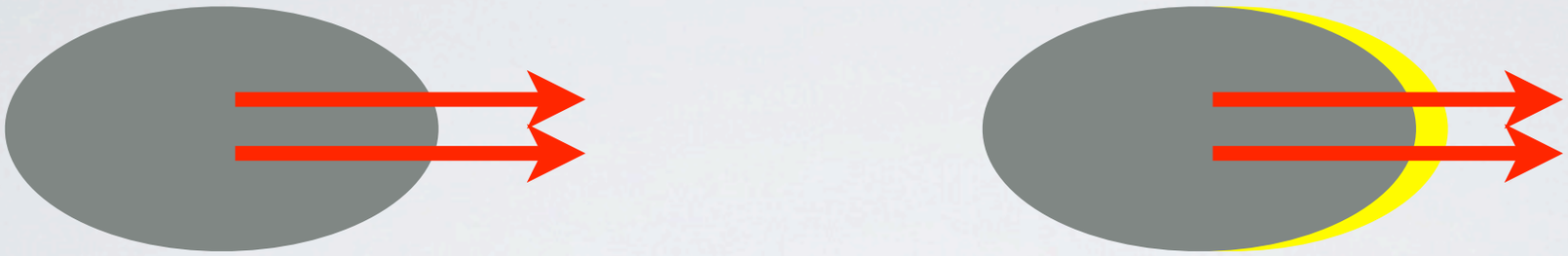


Non-muscle Myosin II: Generates different forces depending on drag coefficient (sliding rate).

Cytoskeleton: Forms filaments (stress fibers) depending on balance between intrinsic time scale and turnover. Intrinsic time scale depends on myosin force

JOHNS HOPKINS
UNIVERSITY

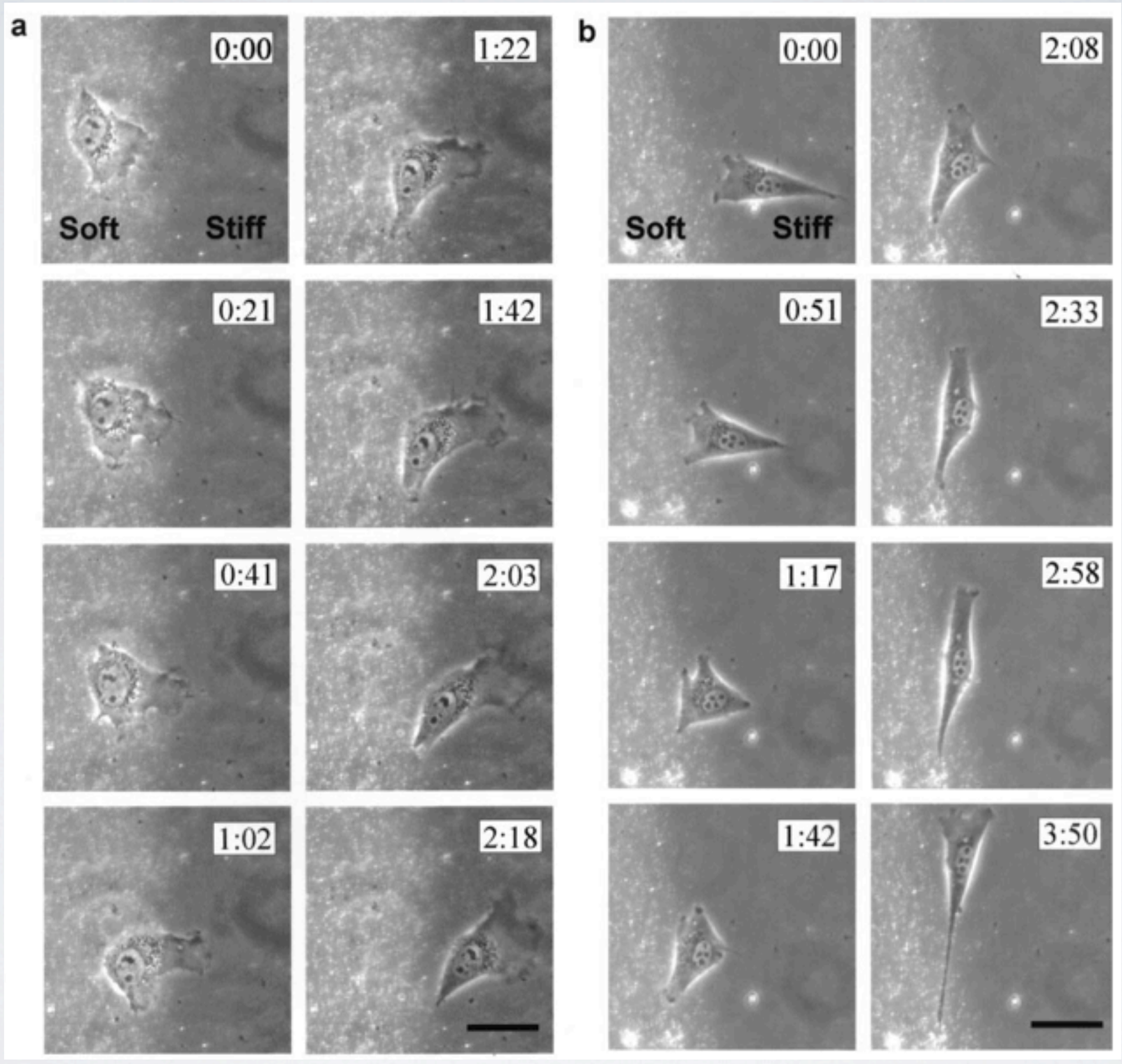
Alternative Models (Geiger, Bershandsky, Safran, Kozlov et al)



Adhesion molecules are static, but adhesion patch grows in the leading edge in the direction of force, and shrinks at the trailing edge. The time scale is governed by adhesion growth.

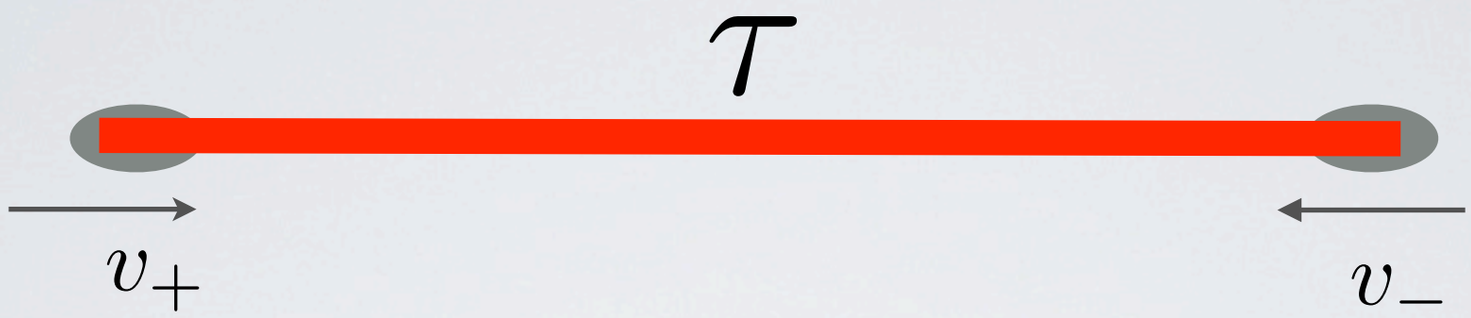
JOHNS HOPKINS
UNIVERSITY

Durotaxis: Migration following stiffness gradients



JOHNS HOPKINS
UNIVERSITY

Friction model can explain movement toward high stiffness



Introduce substrate stiffness gradient:

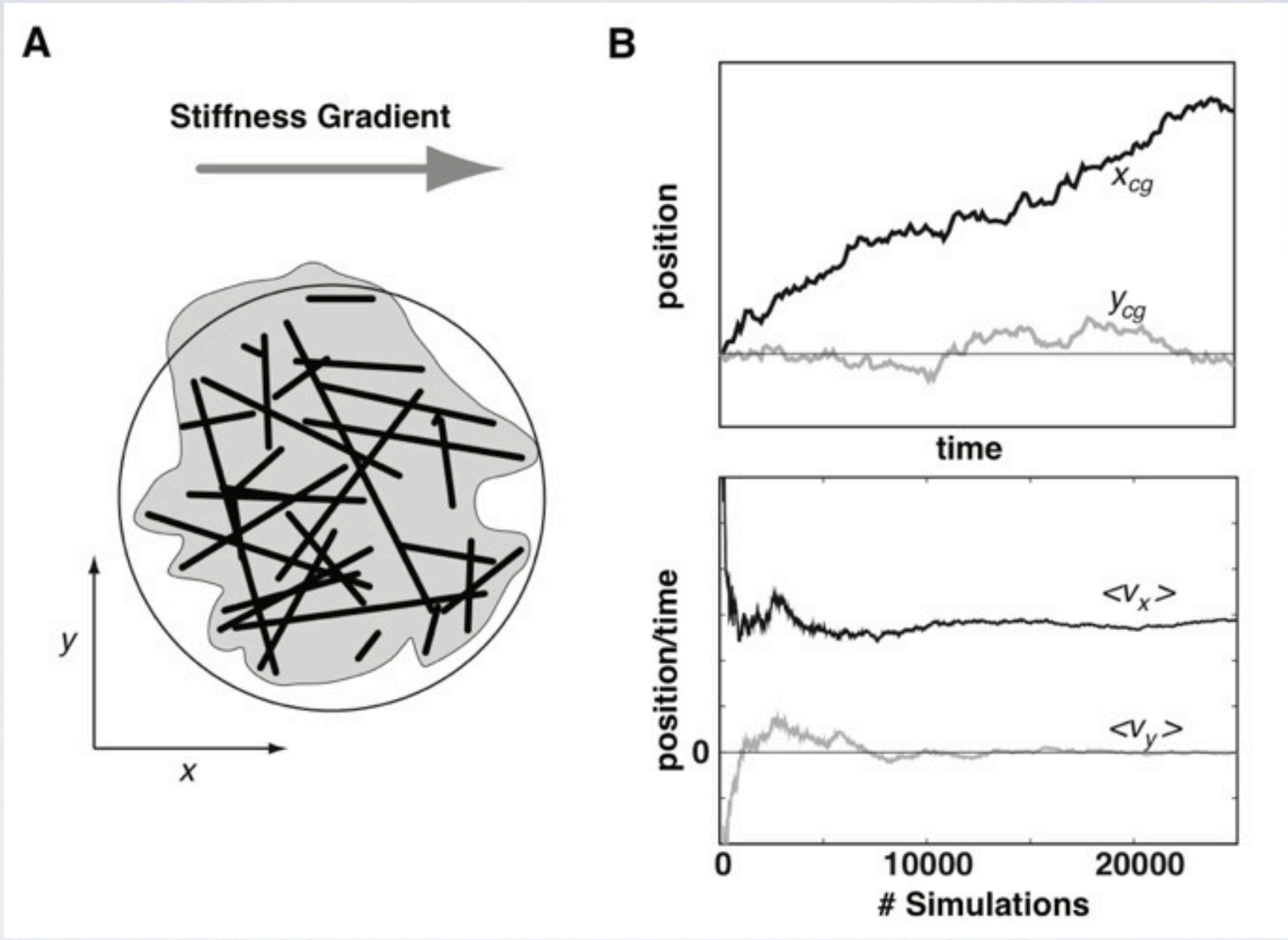
$$E = \nabla E x + E_0$$

$$v_{\mathrm{cm}} = \frac{1}{2}\left(v_+ + v_-\right) = \tau\left(\frac{1}{b_+} - \frac{1}{b_-}\right) \propto \frac{(\nabla E)^2}{E_0}$$

Unbalanced frictional force leads to movement, whose velocity goes down as the absolute value of the substrate elasticity.

JOHNS HOPKINS
UNIVERSITY

Rough whole cell model



Stochastic formation of new stress fibers around the current cell location, with rate that depend on local stiffness. $k(E)$

Contraction of the fibers under constant force τ

JOHNS HOPKINS
UNIVERSITY

Conclusions

- Focal adhesion movement resembles frictional contact.
- Adhesion drag and frictional interaction between cytoskeletal filaments generate bundling dynamics.
- Competition between time scales give rise to observed stress-fiber bundles.
- Simple friction model can explain durotaxis.

Walcott & Sun, PNAS, 2010
Harland, Walcott & Sun, Phys. Biol. 2011

JOHNS HOPKINS
UNIVERSITY

Thank you!