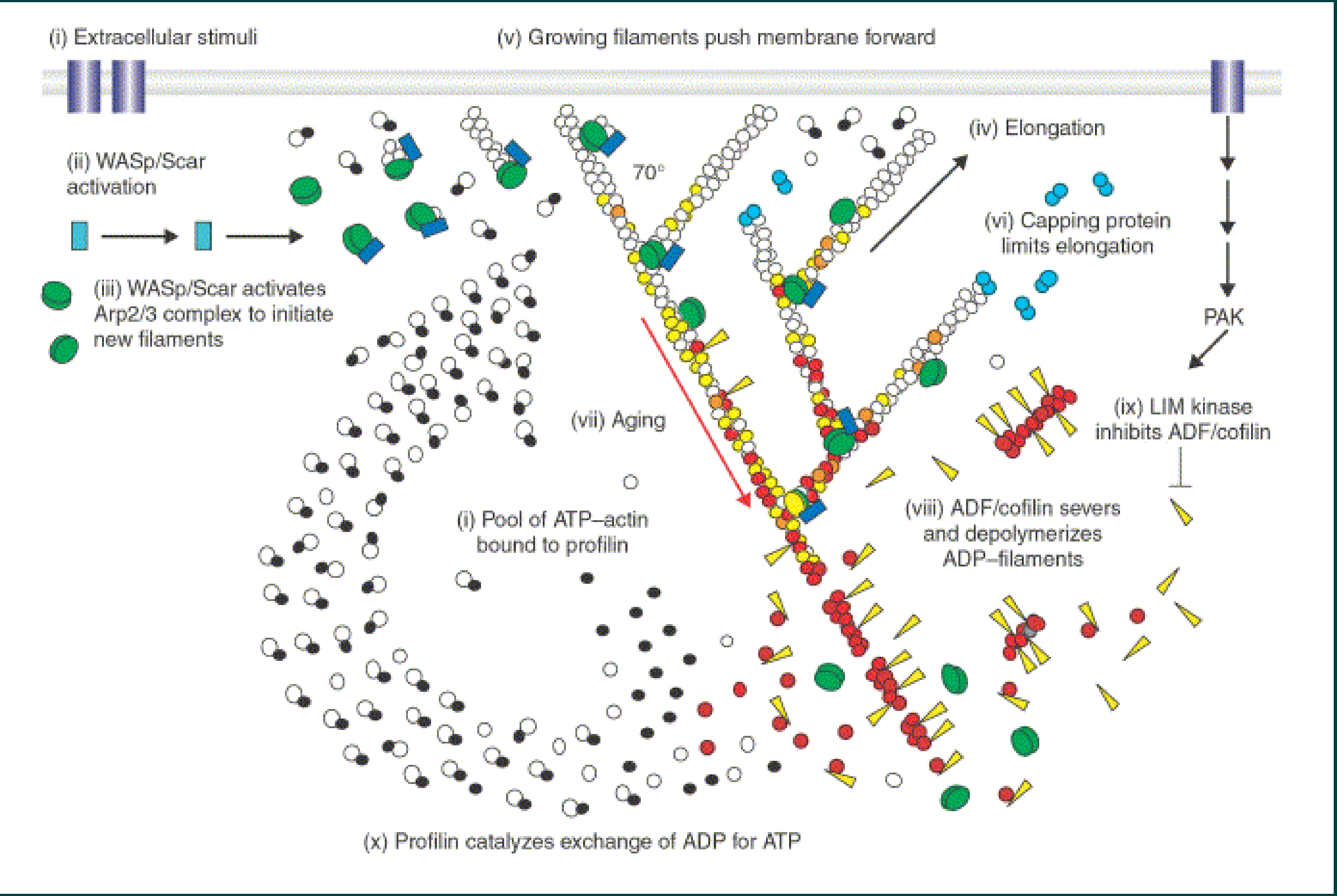
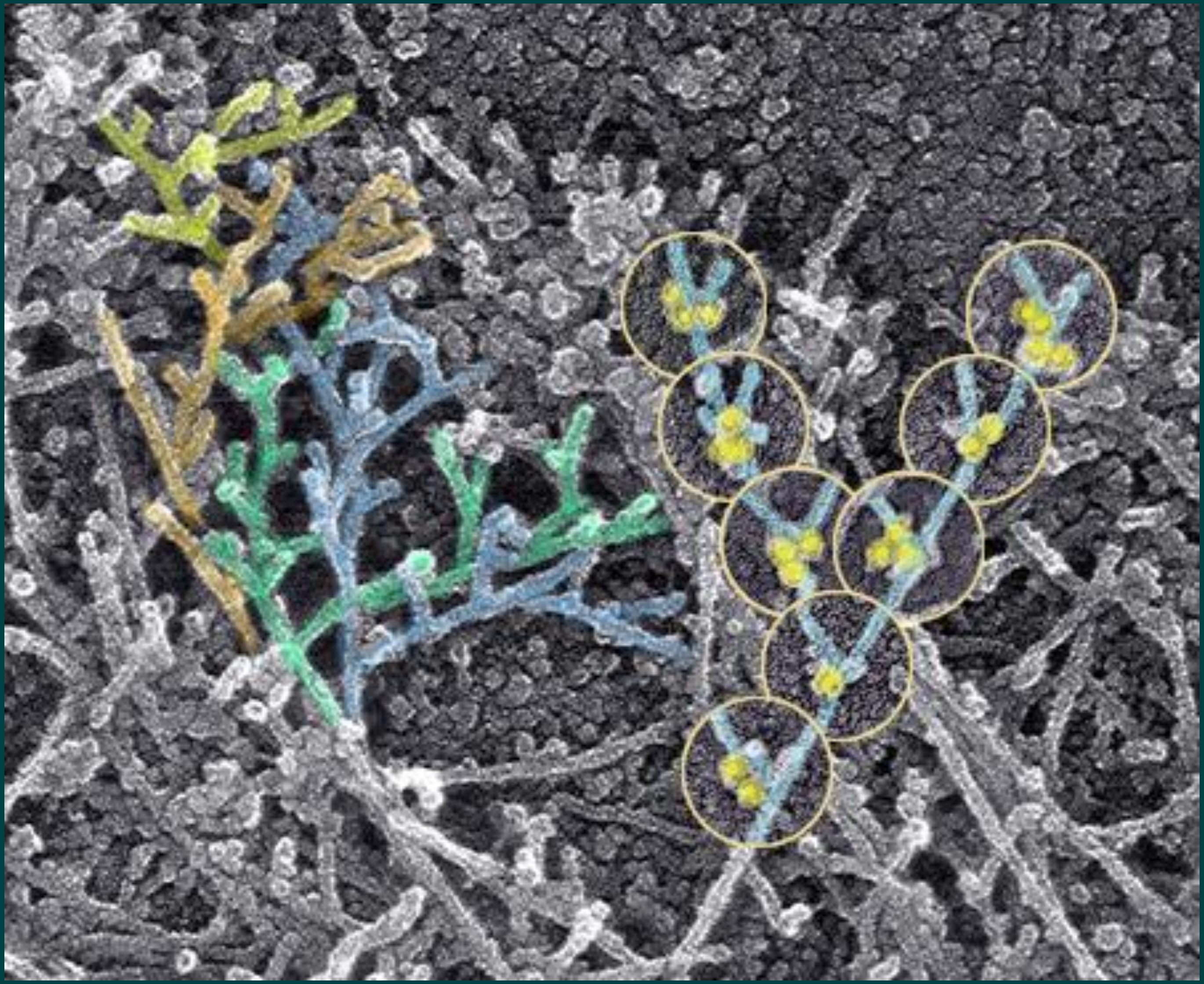
last time: Dendritic nucleation model of actin assembly in the lamellipodia



branching actin network



Svitkina and Borisy, 1999

today:

reconstitution of force generation by branching actin array in vitro

other models of force generation by actin assembly

experimental force measurement in vitro and in vivo

properties of actin branching array in vivo

Reconstitution of actin-based movement from pure proteins (Loisel et al., Nature 1999)

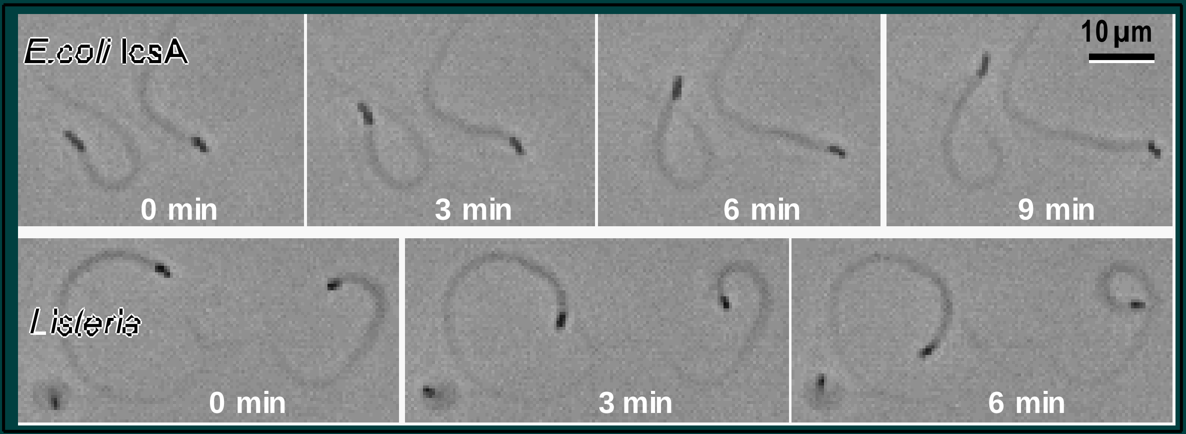
- Proteins required for movement:

1) N-WASP (resp. ActA)-activated Arp2/3: site-directed generation of barbed ends

2) Actin, ADF/cofilin,Capping protein: chemostat maintaining a high steady-state concentration of ATP-G-actin

- Not required, but improve movement:
  - VASP, Profilin, α-actinin.

Movement of *E. coli* (IcsA) and *Listeria monocytogenes* with pure components.



Rate :
2 to 3 µm/min

Essential Proteins :

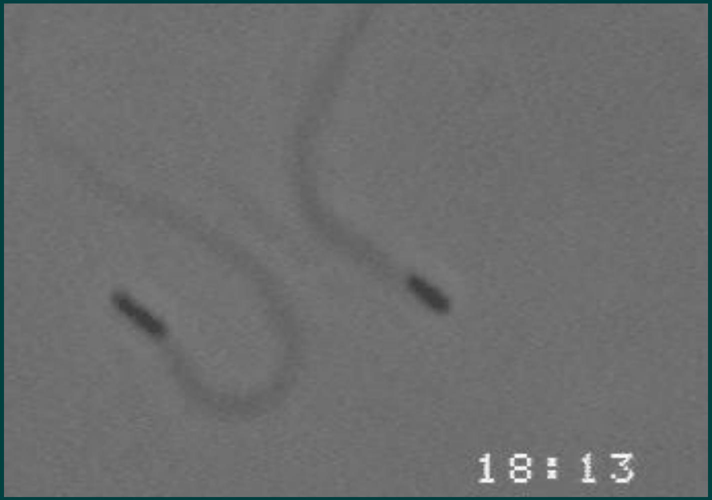
N-WASP	IcsA-bound
Arp2/3	0.1 µM
Capping Protein	0.1 µM
ADF	2 µM
ATP-actin+F-actin	8 µM

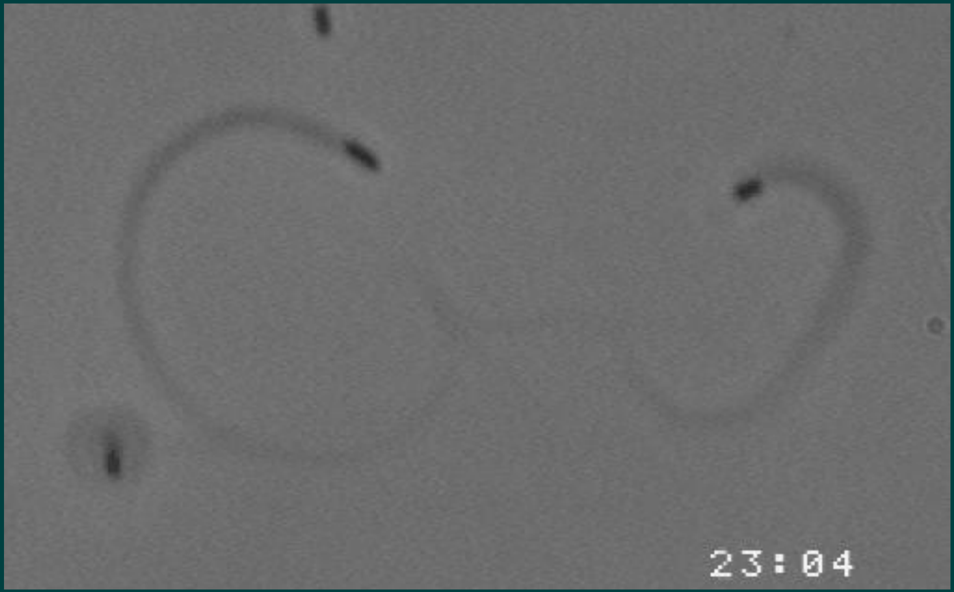
Useful Proteins :

Profilin	2 µM
α-actinin	0.5 µM
VASP	0.1 µM

Movement of *E. coli* (IcsA) and *Listeria monocytogenes* with pure components.

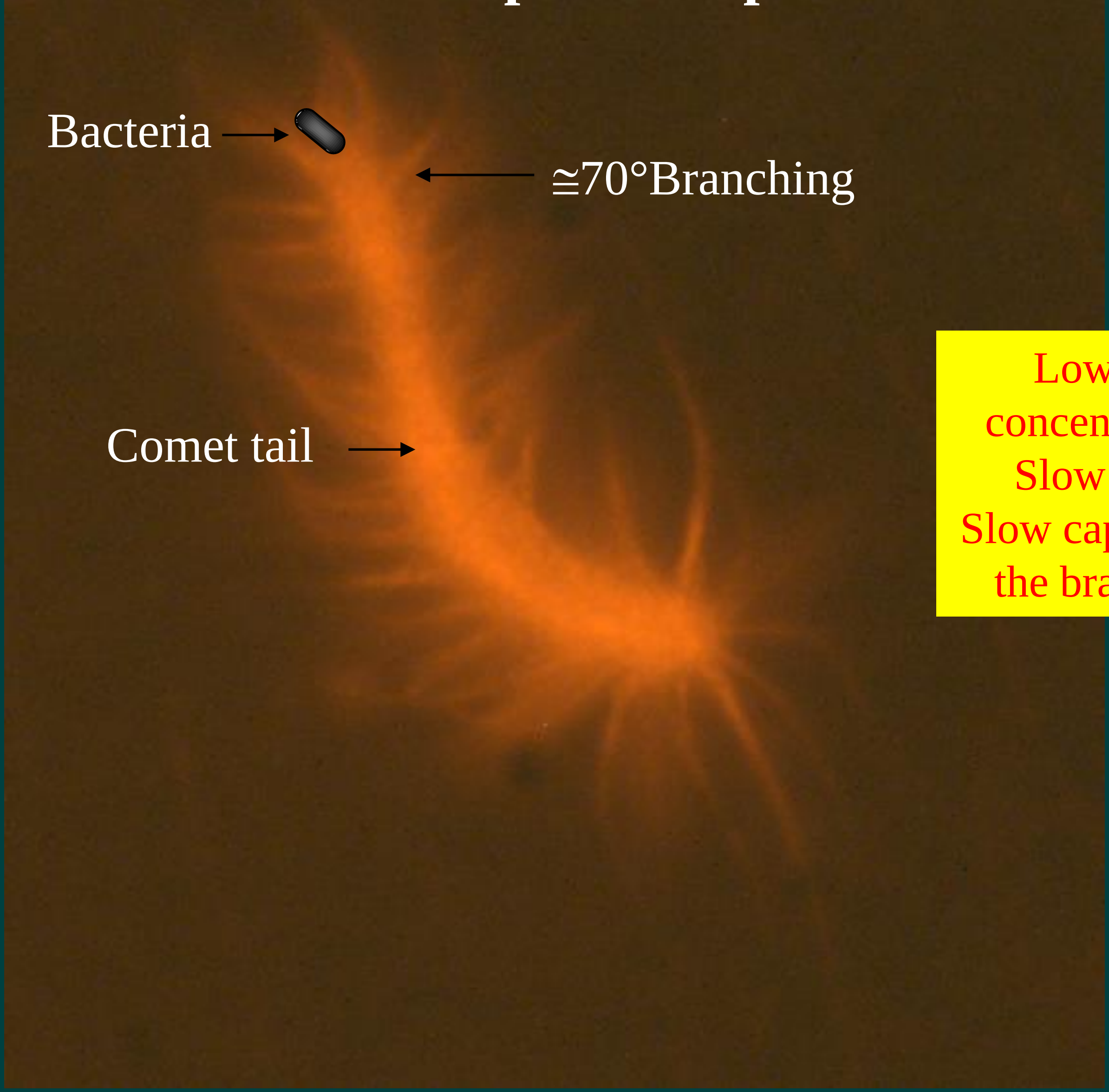
E. coli IcsA
Rate :
≈2 µm/min





Listeria
Rate :
≈3 µm/min

Movement of *E. coli* (IcsA) and *Listeria monocytogenes* with pure components.

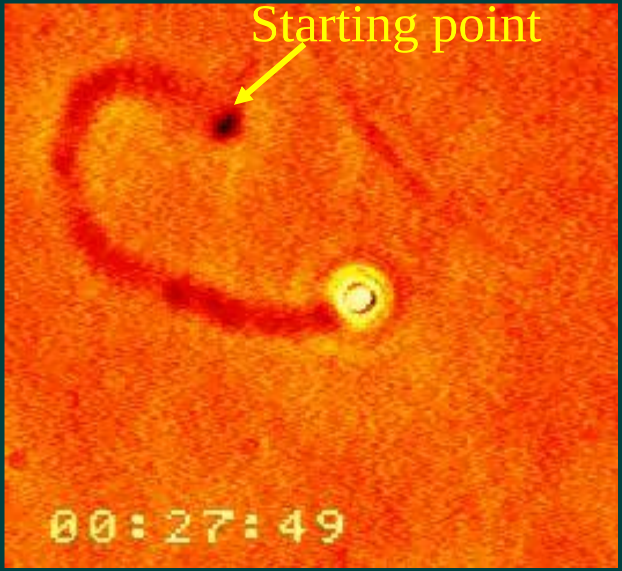


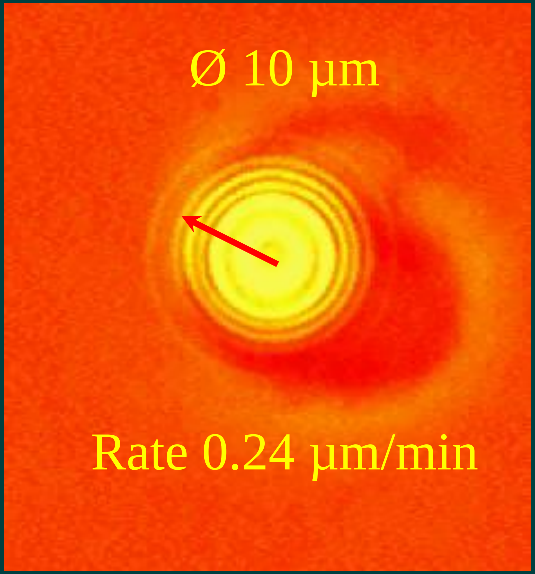
Low CP concentration
Slow Rate
Slow capping of the branches

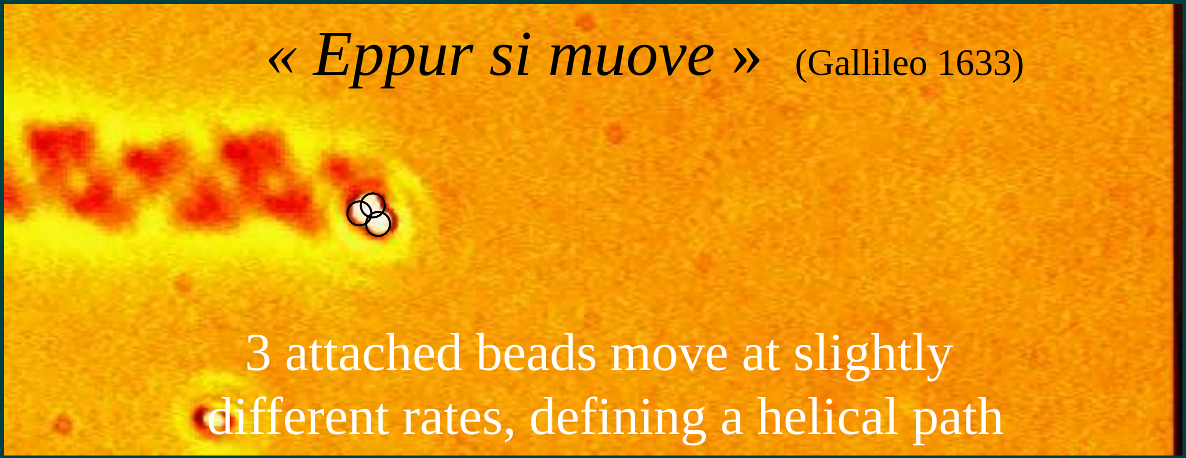
Movement of *E. coli* (IcsA) and *Listeria monocytogenes* with pure components.

Low CP concentration
Slow Rate : less than
1 µm/min
Poor capping of
the branches

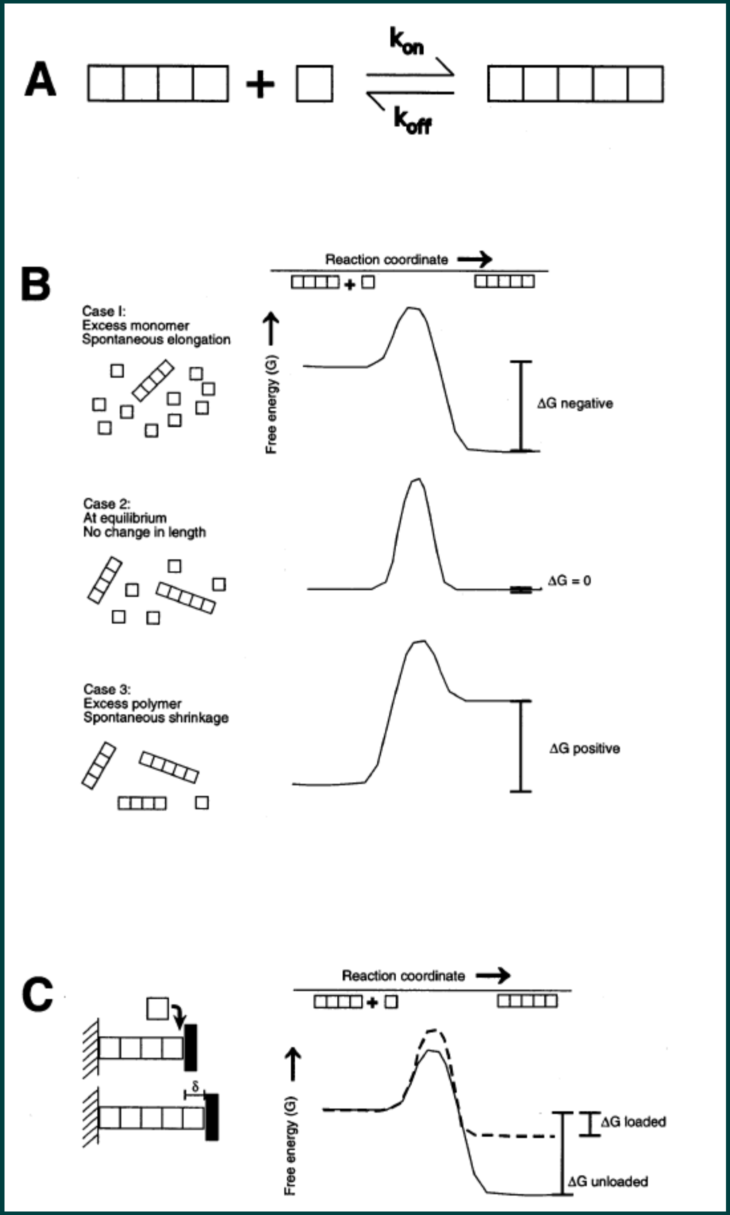
Any object N-WASP-coated can move in minimal motility medium







thermodynamics of force generation by filament assembly



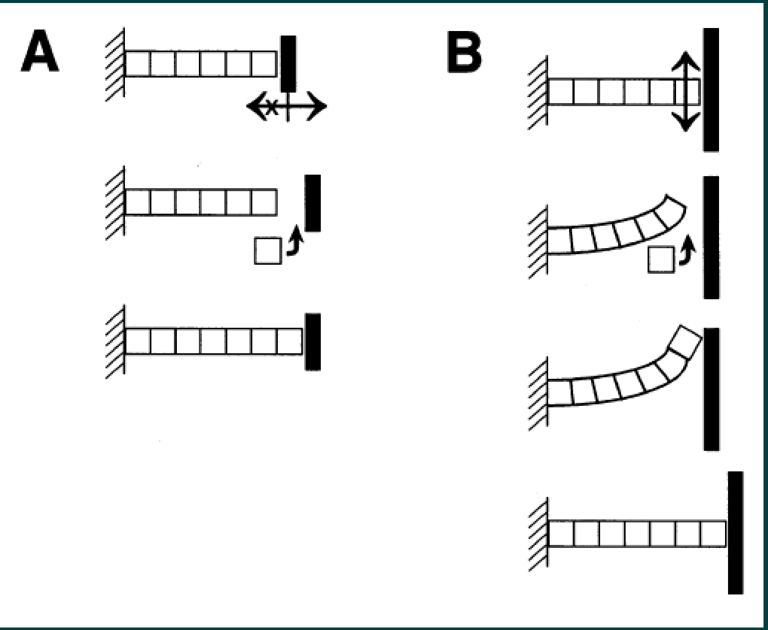
$$F_{max} = \frac{k_B T}{\delta} \ln \frac{m}{m^*}$$

$$m^* = K_d(0)$$

$$F_{max} = \frac{4 \text{ pN nm}}{2.7 \text{ nm}} \times \ln 100 \approx 7 \text{ pN}$$

Theriot, 2000

Polymerization Brownian ratchet



Theriot, 2000

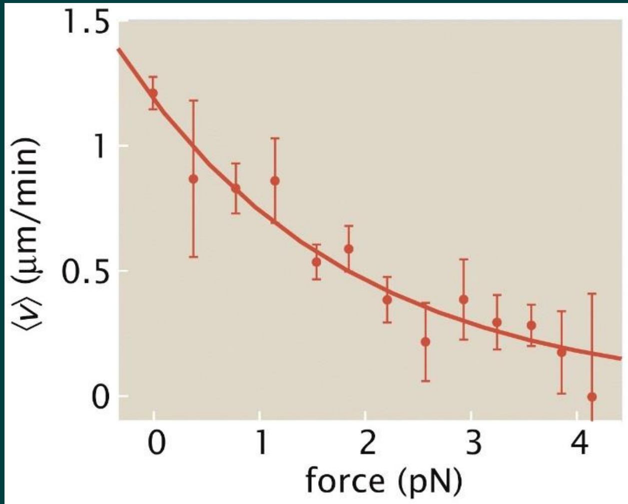
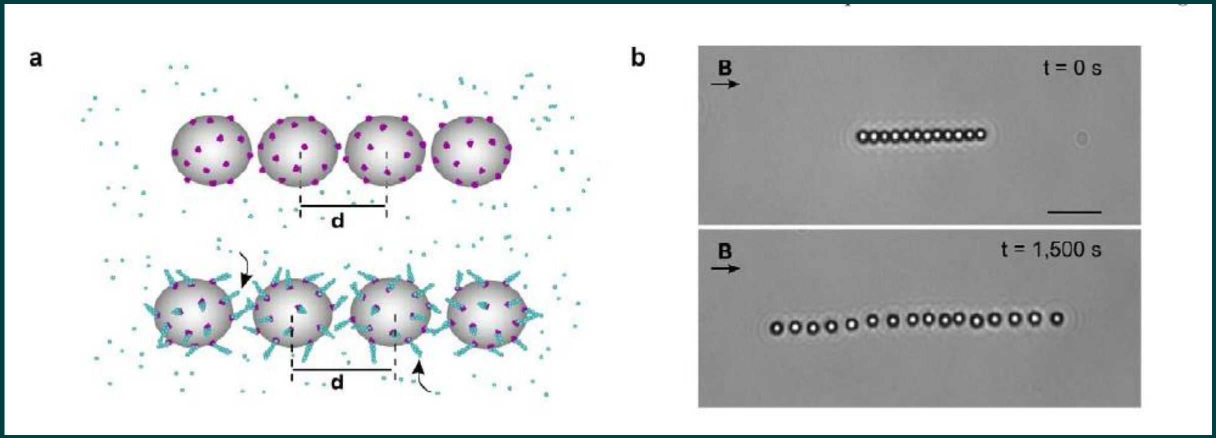
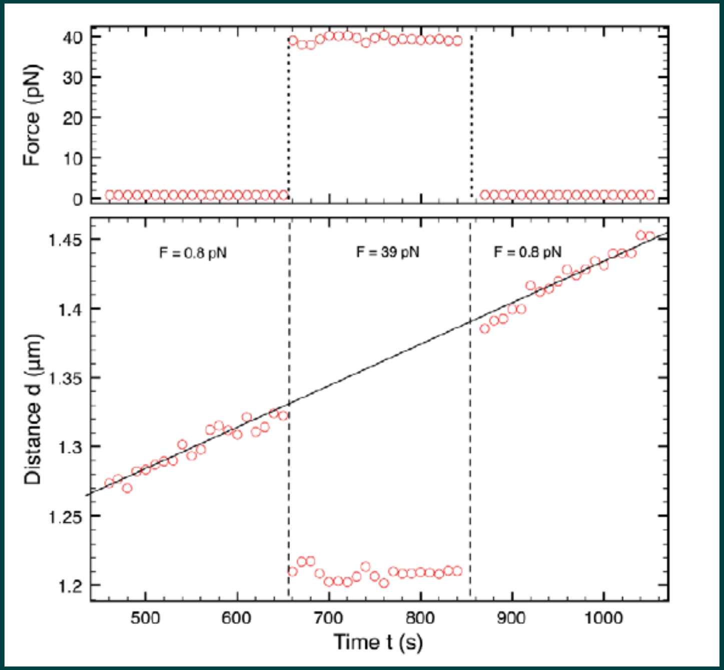


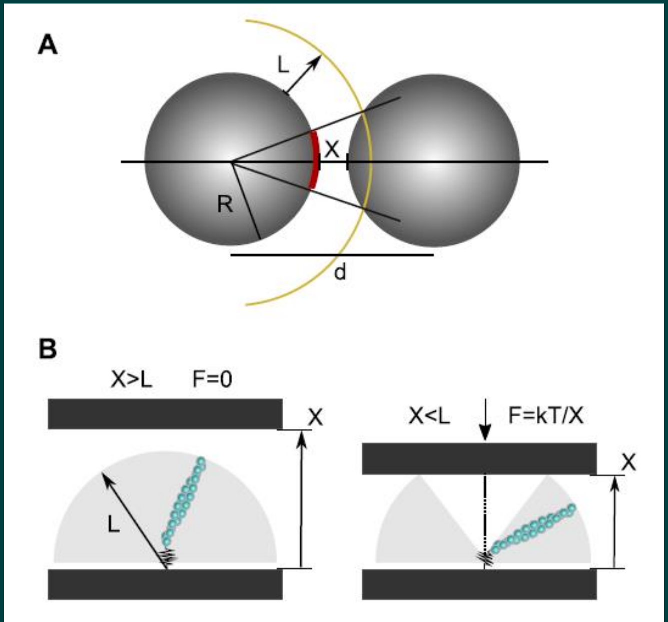
Figure 16.48 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

$$v = \delta[k_{on} m e^{-F\delta/k_B T} - k_{off}]$$

when filaments are flexible, force deflects them bu doesn't slow their growth (inconsistent with Brownian ratchet)

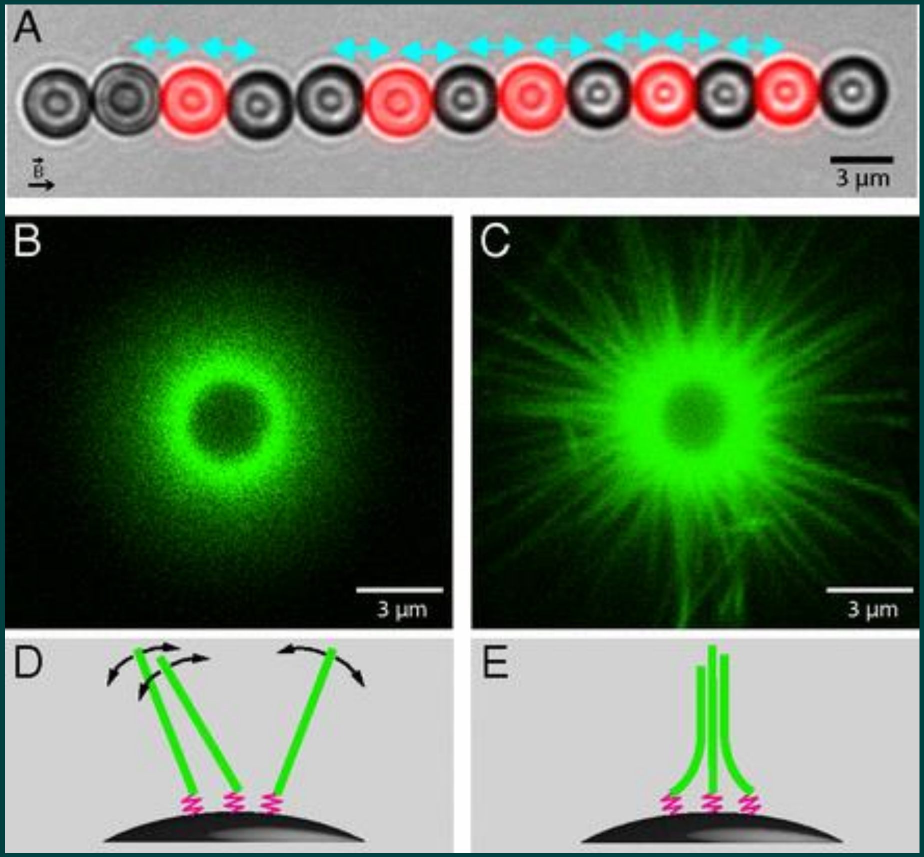






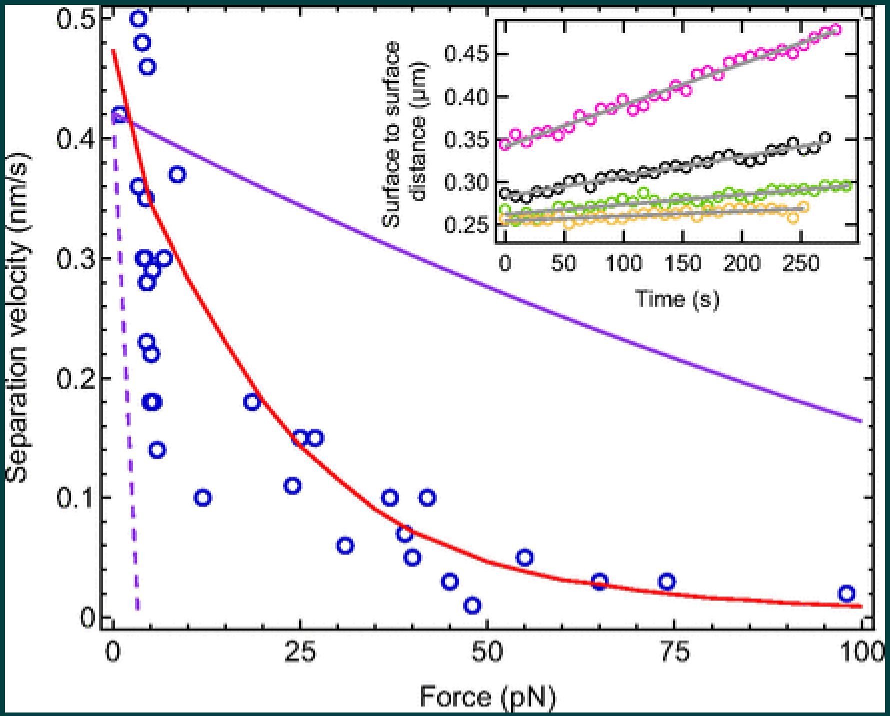
Brangbour et al., 2011

Force production by rigid filament bundles is consistent with Brownian ratchet mechanism



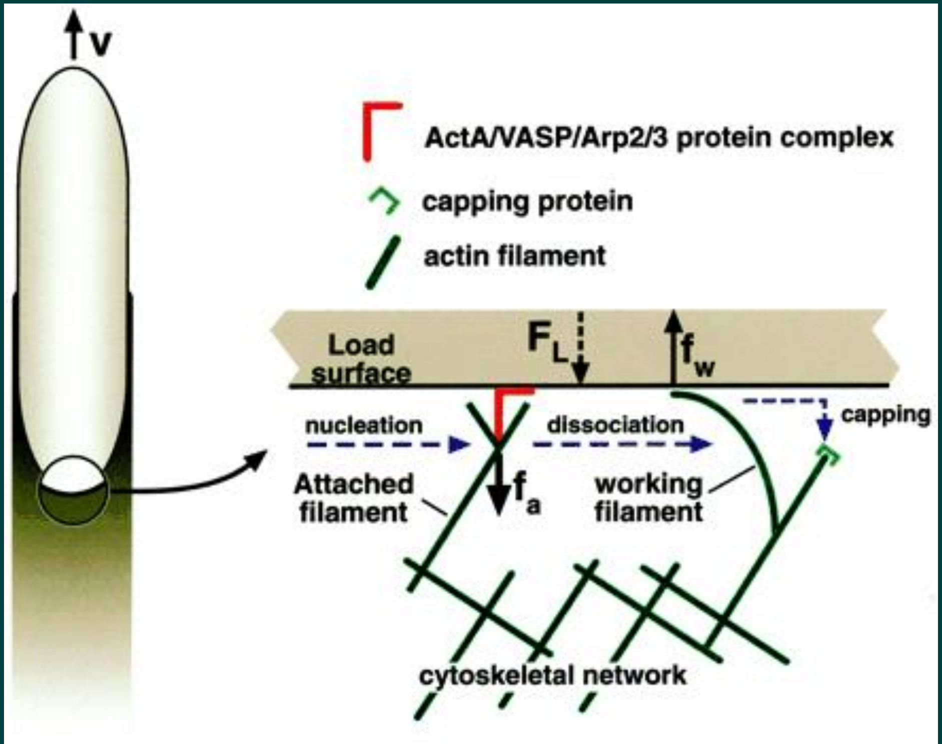
no bundling

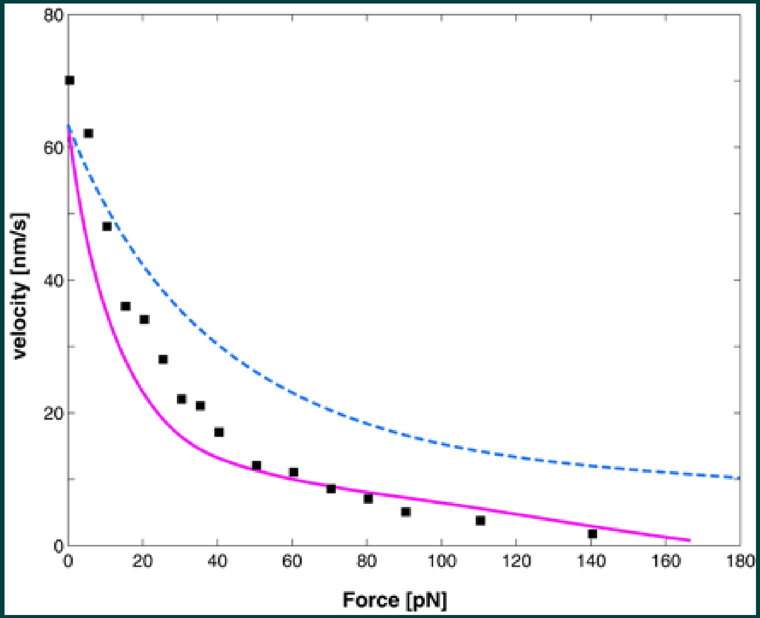
bundling with fascin



Démoulin et al, 2014

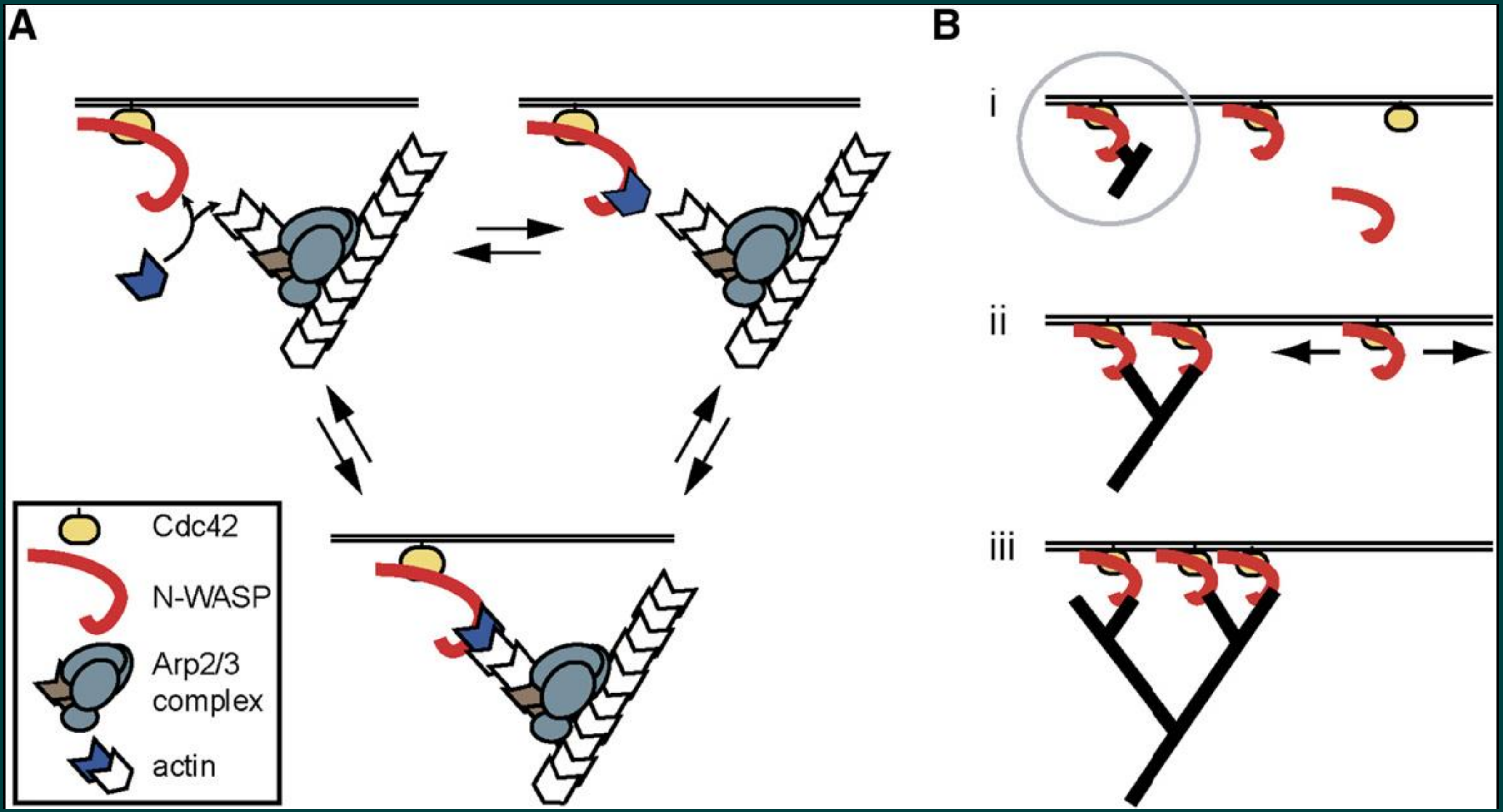
tethered Brownian ratchet: pushing and attached filaments



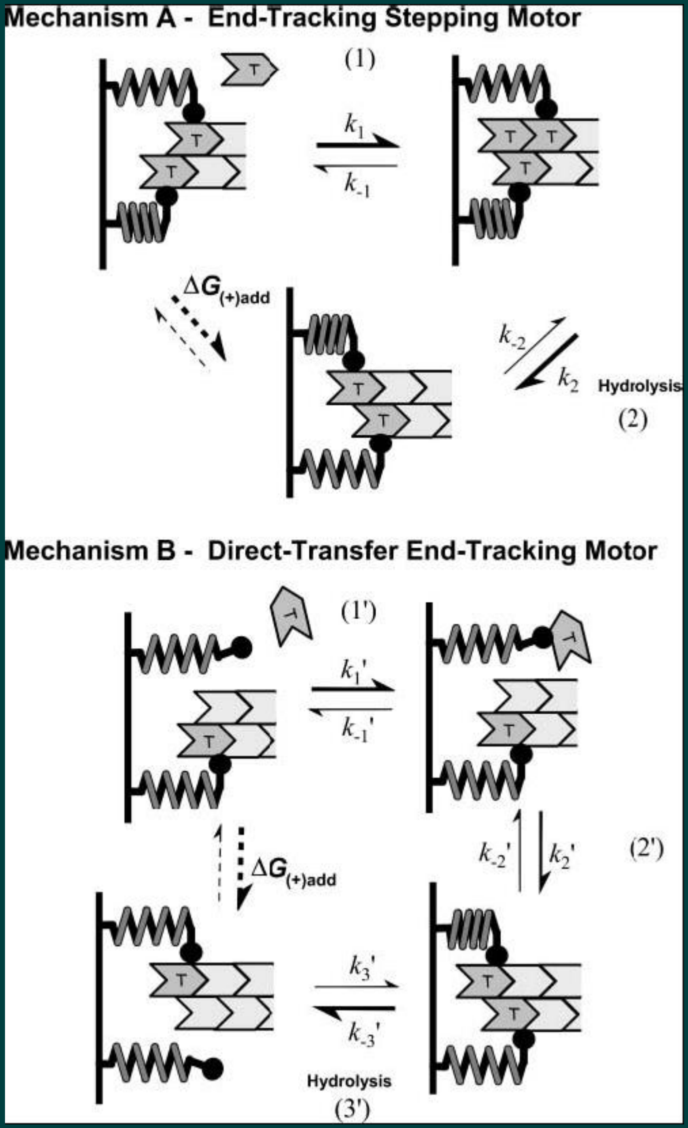


Mogilner, A. & Oster, G. (2003) *Biophys. J.* **84,** 1591–1605.

N-WASP attaches barbed filament ends to the membrane
Co et al., Cell, 2007

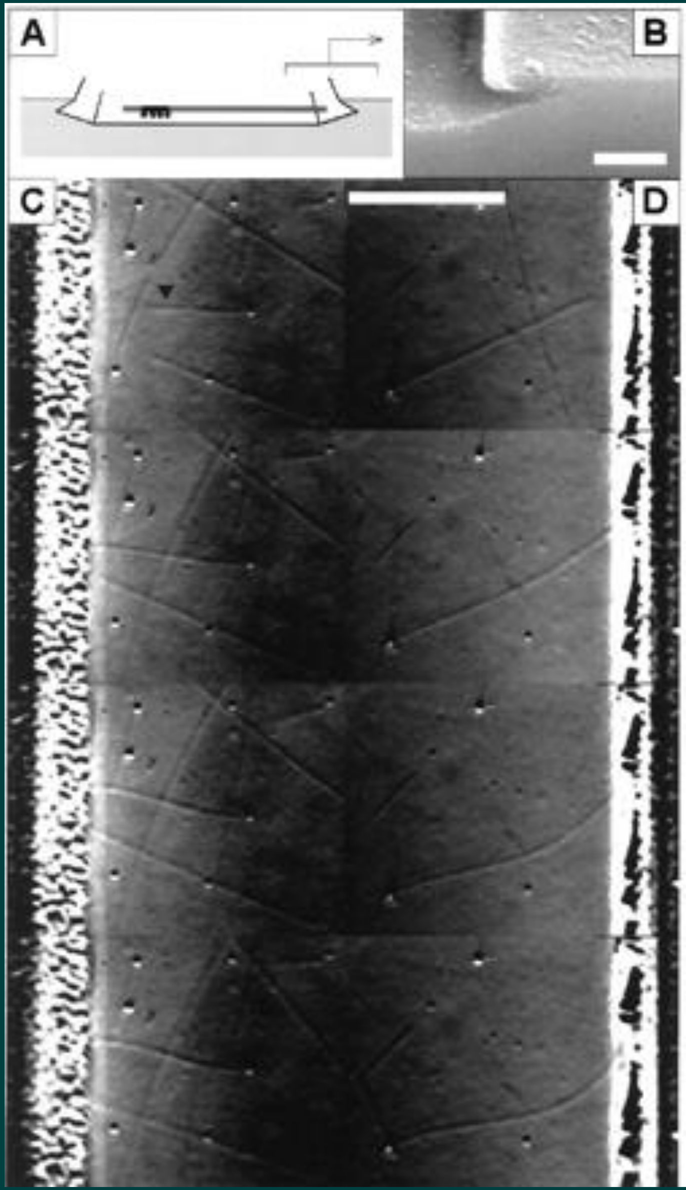


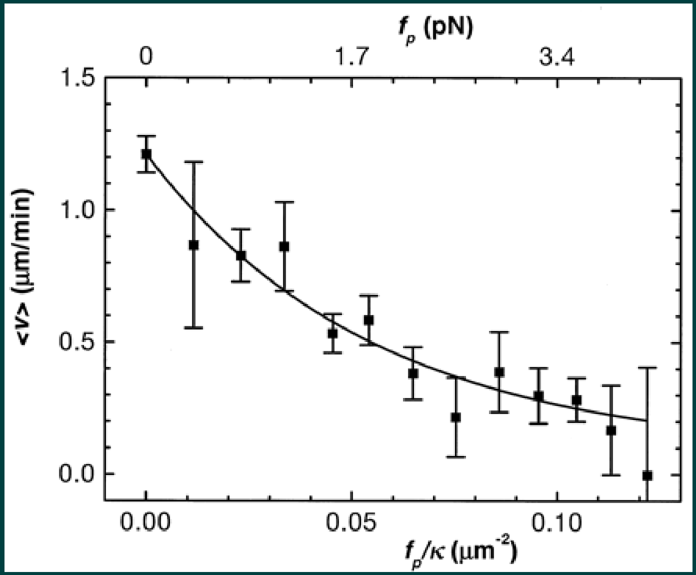
Alternative to Brownian ratchet – end-tracking motor



Dickinson et al.,
Biophys. J., 2004

Experimental measurement of polymerization force





Force-velocity relation for growing microtubule

$$v(f_p) = \delta[\alpha \exp(-f_p\delta/k_B T)c - \beta]$$

Dogterom M, Yurke B. Science. 1997 Oct 31;278(5339):856-60.

with optical trap for microtubules

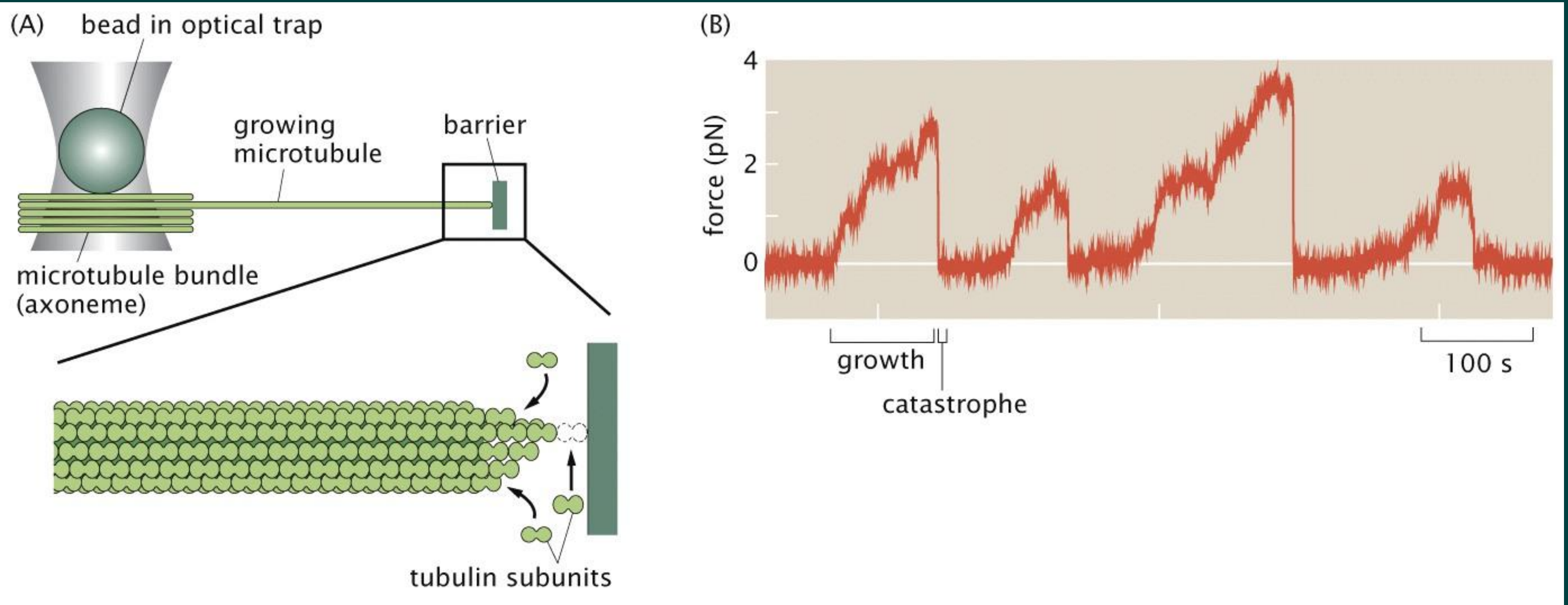
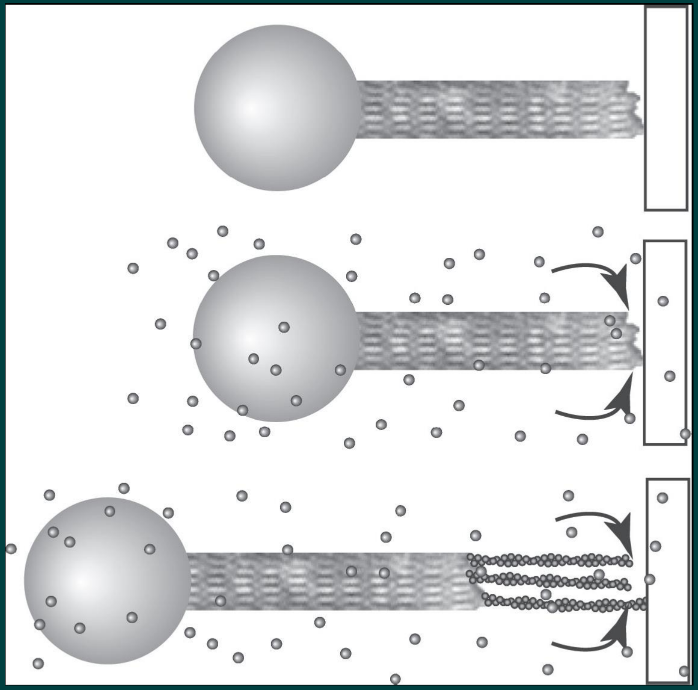
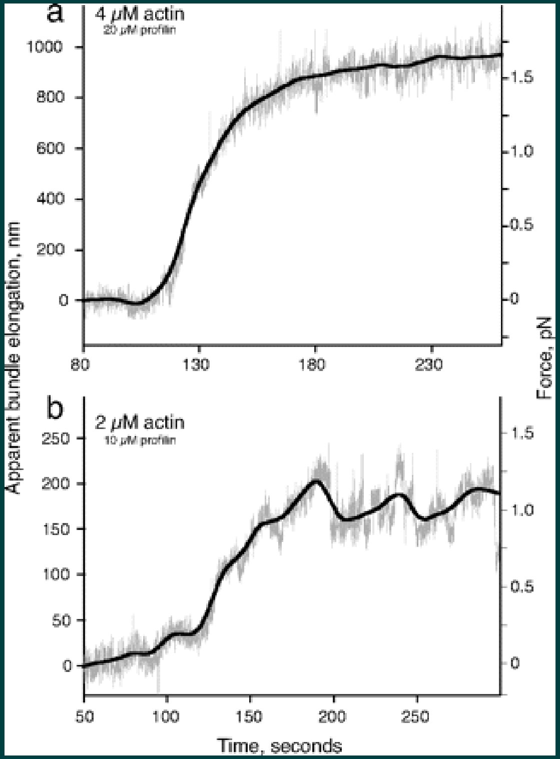


Figure 16.49 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

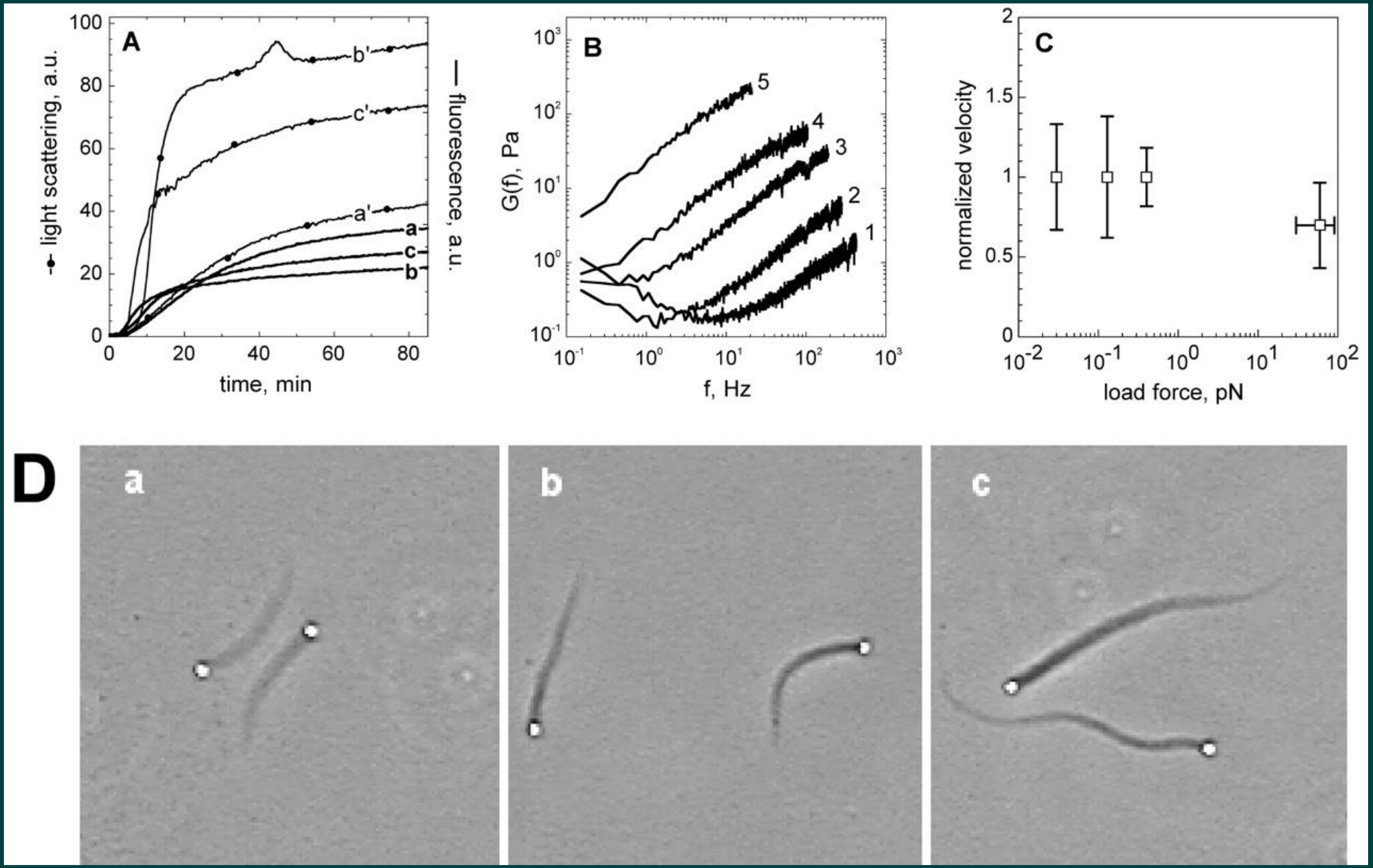
with optical trap for actin





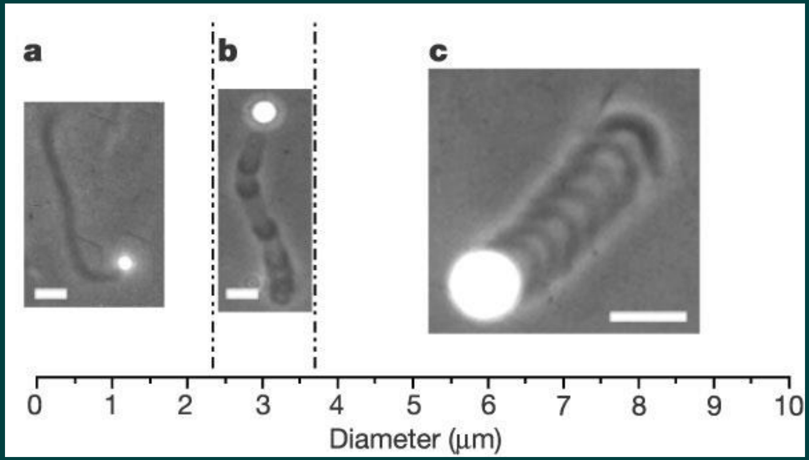
Footer et al., PNAS, 2007

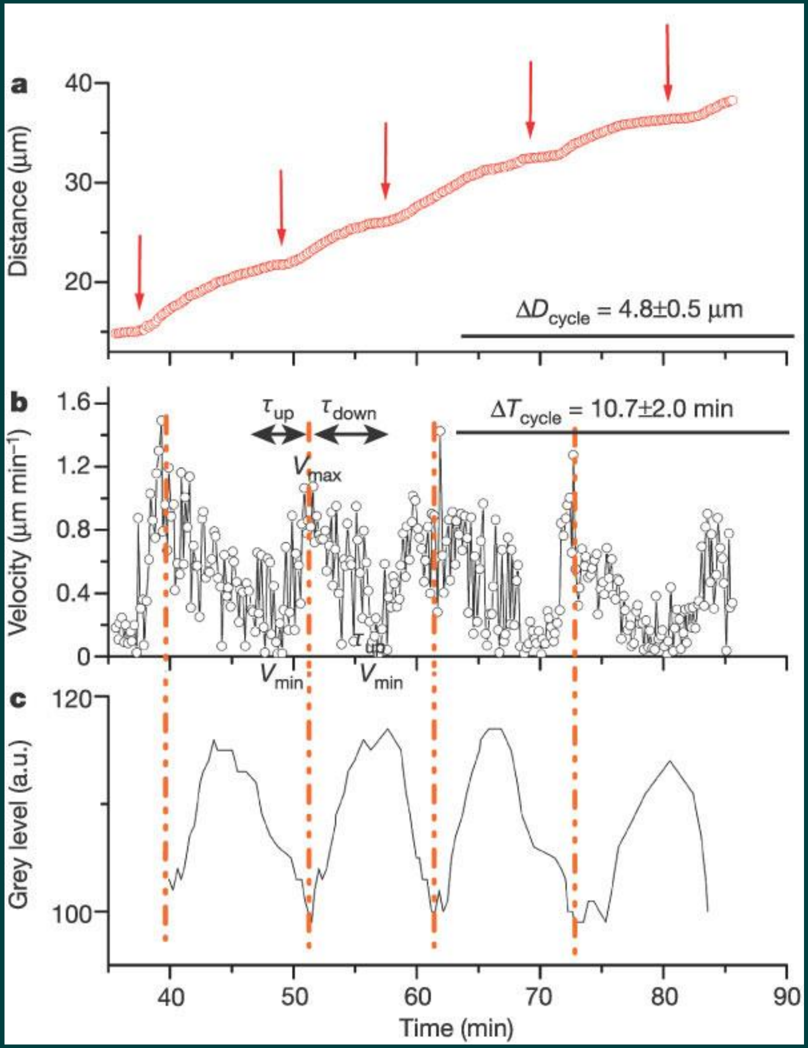
assembly of many filaments: velocity independent of viscous drag up to 50 pN



Wiesner, S., E. Helfer, D. Didry, F. Lafuma, M.-F. Carlier, and D. Pantaloni. 2003. *J. Cell Biol.* 160:387–398

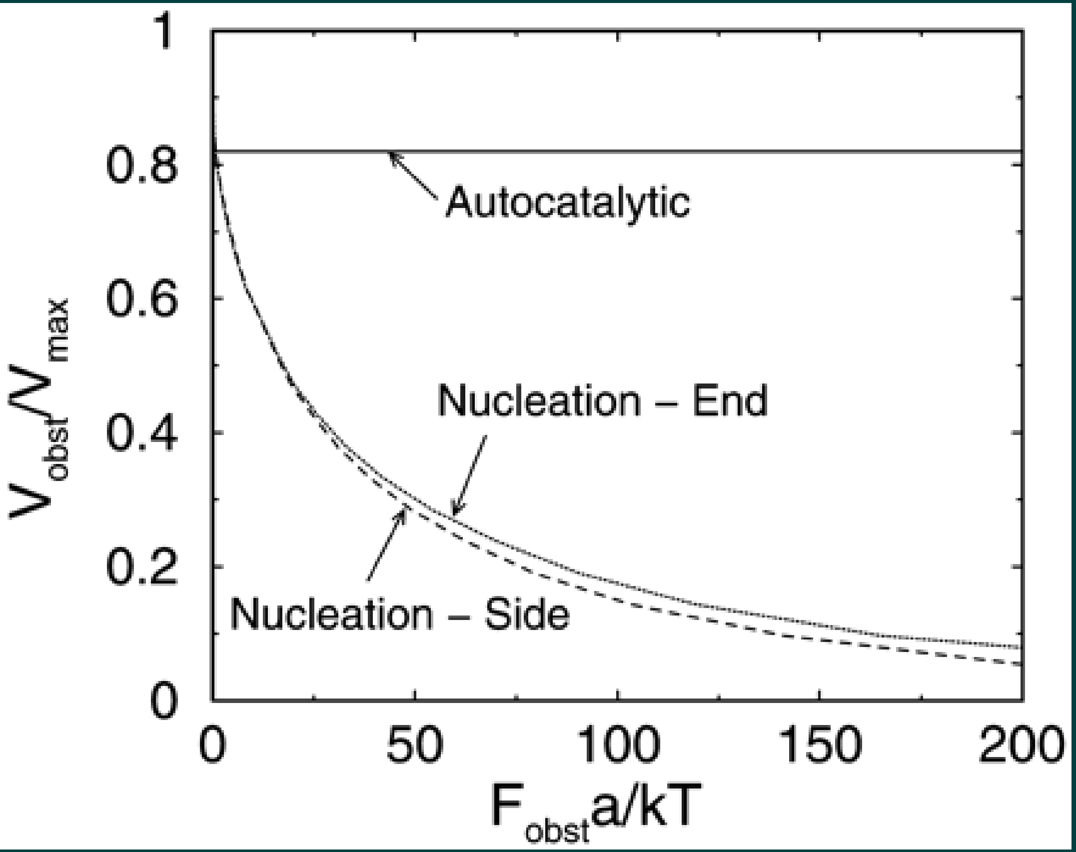
Fluctuations of velocity and tail density





Bernheim-Groswasser, A., Wiesner, S., Golsteyn, R. M., Carlier, M. F. & Sykes, C. (2002) *Nature* **417,** 308–311

Effects of branching kinetics:
autocatalytic branching results
in increase of filament density with force
and flat force-velocity relationship



Carlsson AE. Biophys J. 2003 May;84(5):2907-18.

force-velocity relationship for actin network measured with AFM cantilever

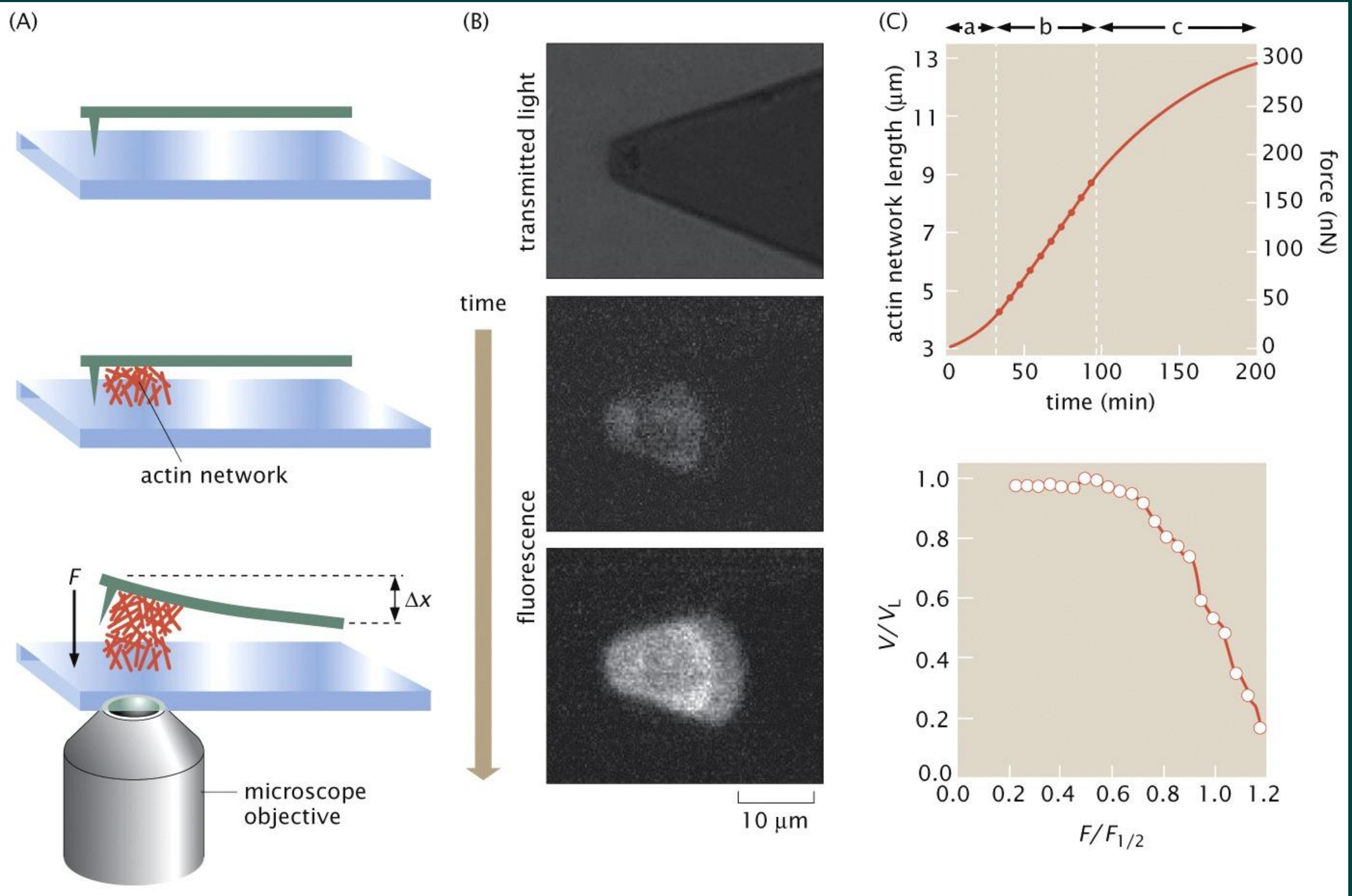
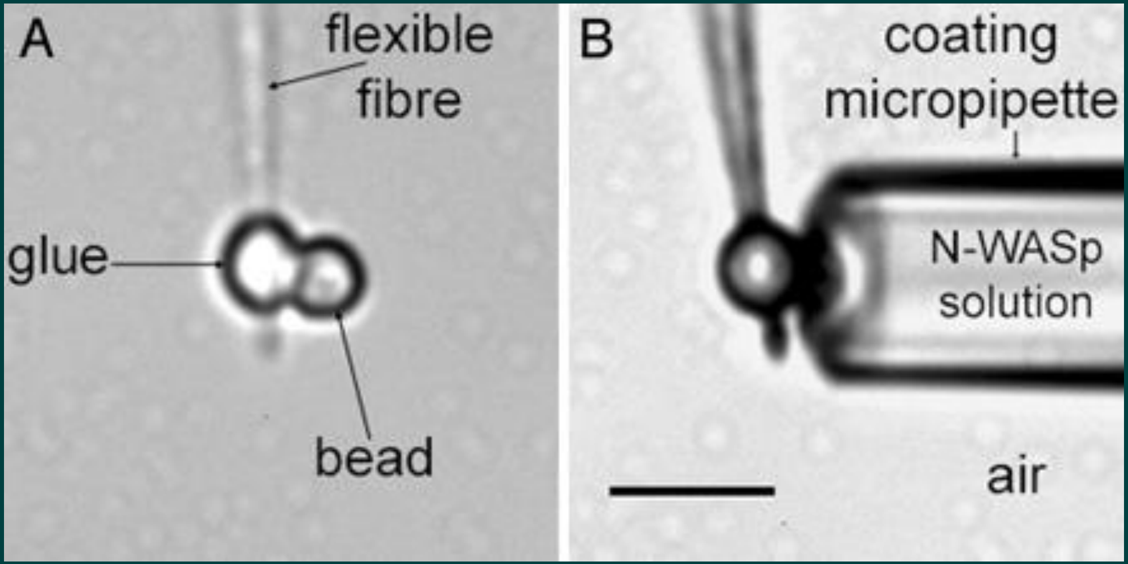
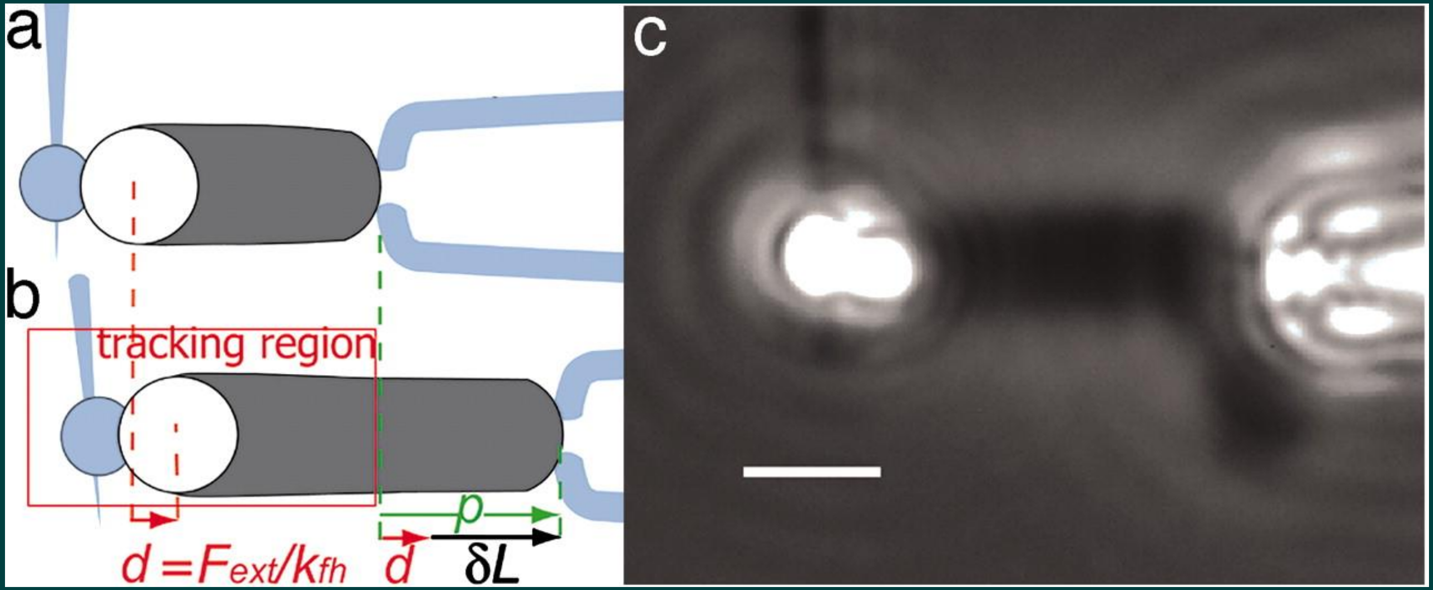


Figure 16.50 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

see also: Bieling et al., Cell, 2016

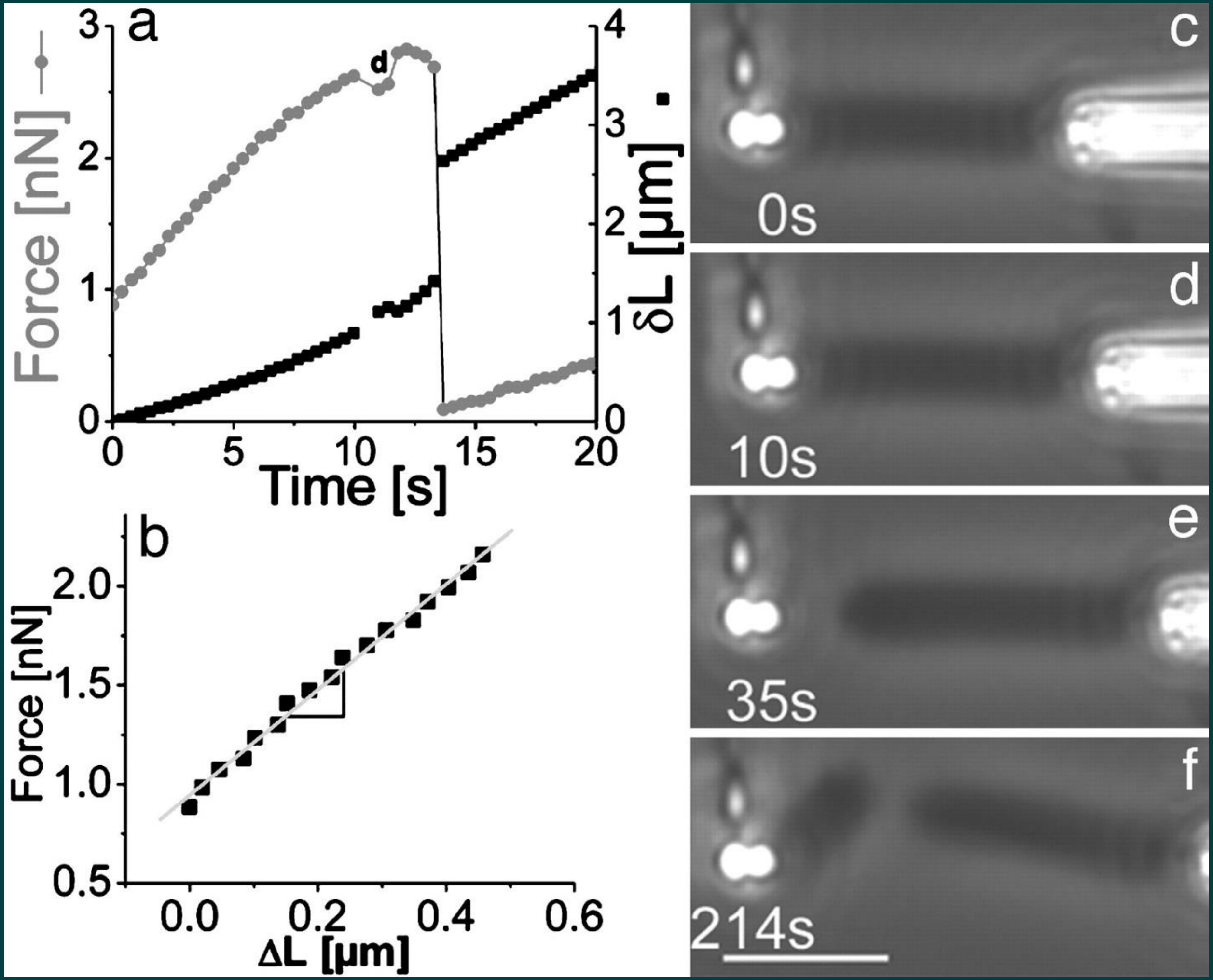
Measurement of force-velocity relation for actin comet-tail with a flexible glass needle





Marcy Y, Prost J, Carlier MF, Sykes C. Proc Natl Acad Sci U S A. 2004 101:5992-7.

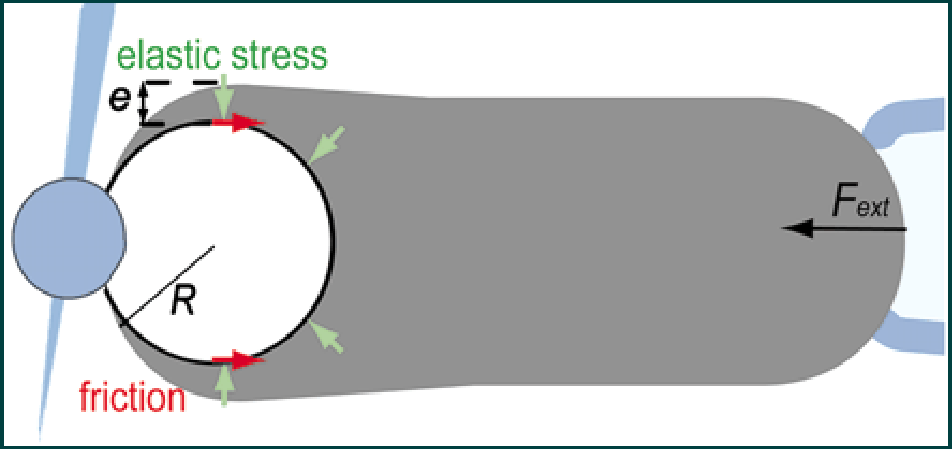
Fig. 3. Fast-pulling, detachment, and regeneration of a comet



force-extension curve allows estimation of elastic modulus

Marcy, Yann et al. (2004) Proc. Natl. Acad. Sci. USA 101, 5992-5997

elastic gel model of force generation

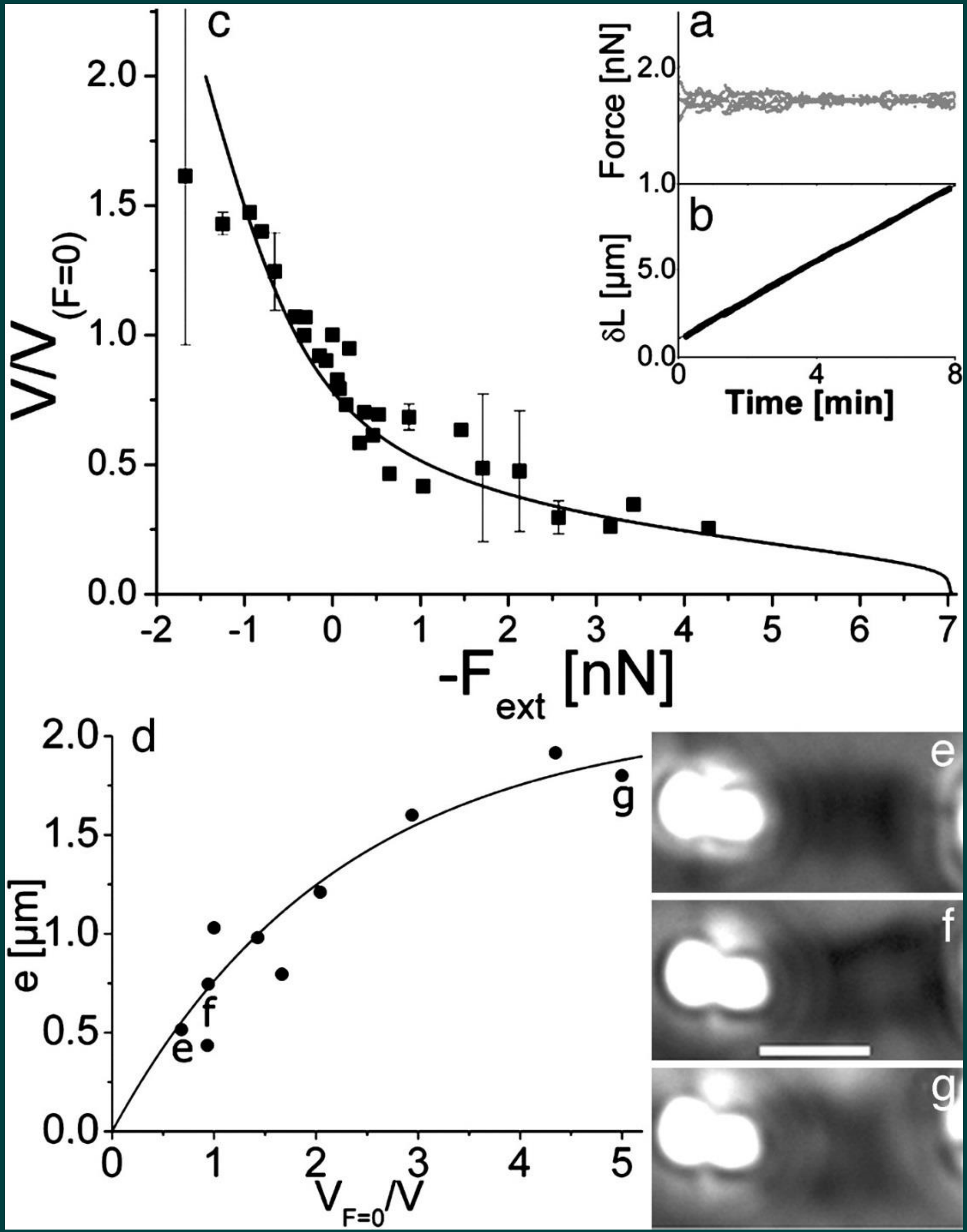


$$F_{\text{ext}} + F_{\text{el}} + F_{\text{fric}} = 0.$$

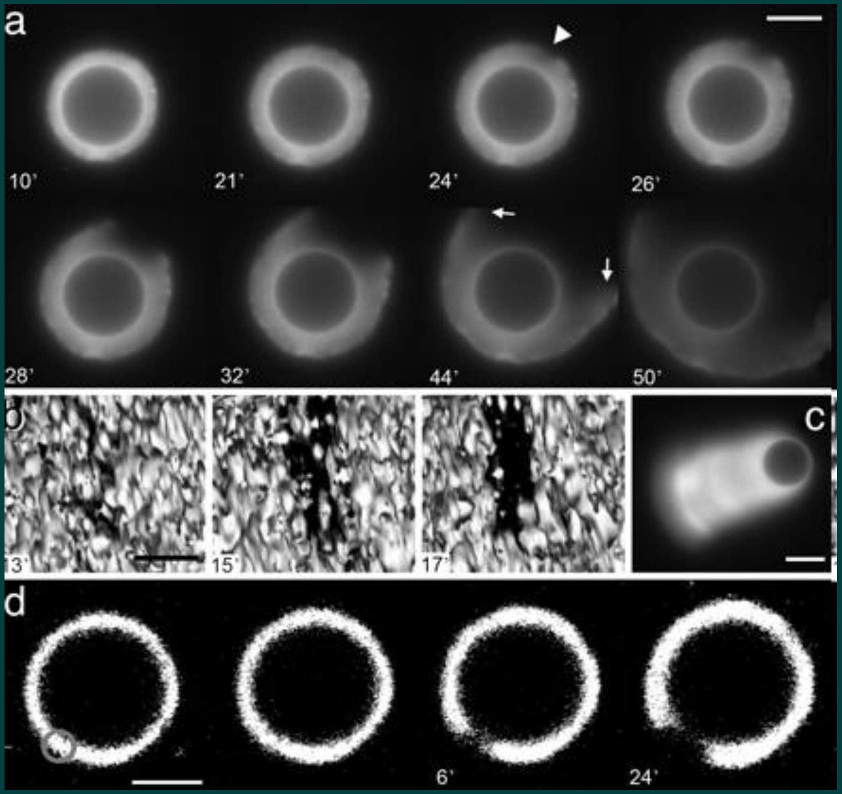
$$F_{\text{ext}} + E\frac{e^3}{R} \simeq S\xi V.$$

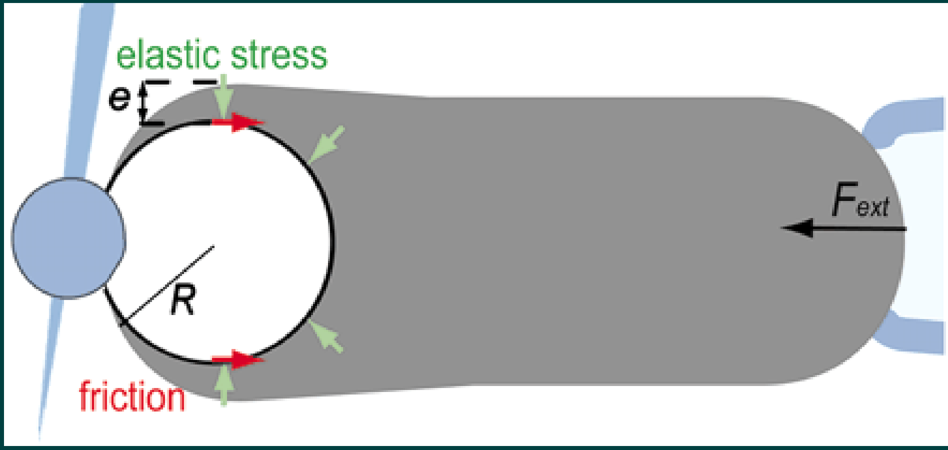
$$e(V) = e^*\left[1 - \exp\left(-\frac{V_p^0 R}{V e^*}\right)\right].$$

Force-velocity relation



Alternative (?) to Brownian ratchet – elastic gel theory
- symmetry breaking and propulsion





Marcy Y, Prost J, Carlier MF, Sykes C. PNAS, 2004; Gocht et al., PNAS, 2005

Are elastic gel and Brownian ratchet models really alternative?

They consider events at different scales: could be complementary to each other (?)

What is the feature that could distinguish between the two models?

Brownian ratchet model implies
that filaments grow with their ends to the surface

In elastic gel theory filament orientation is not important

Brownian ratchet model implies
that filaments grow with their ends to the surface

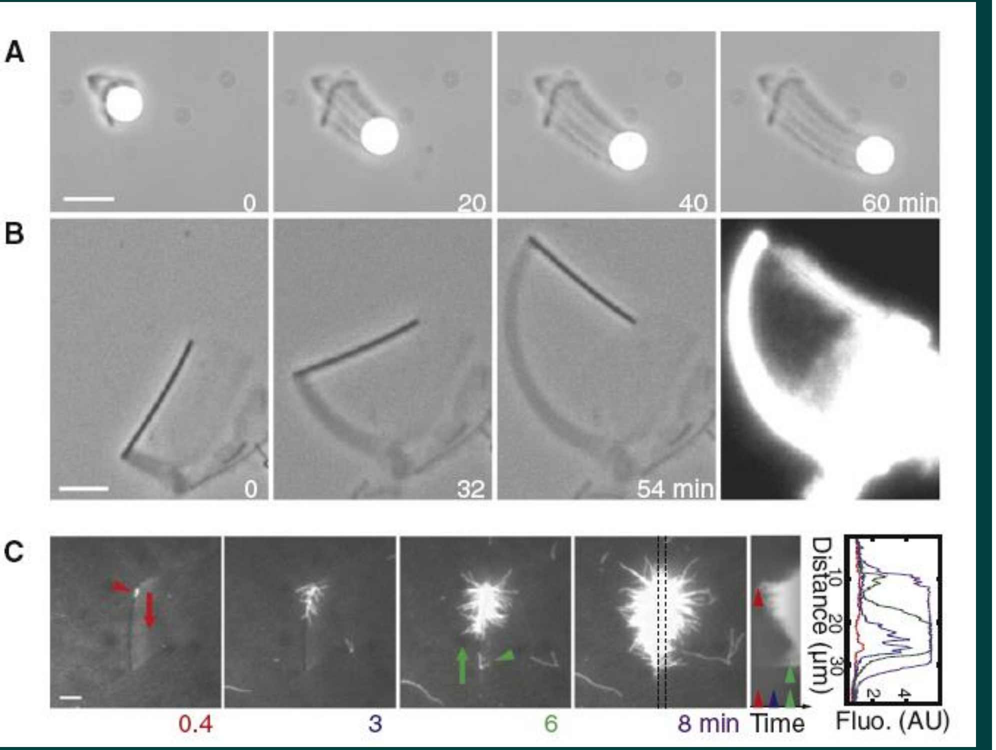
In elastic gel theory filament orientation is not important

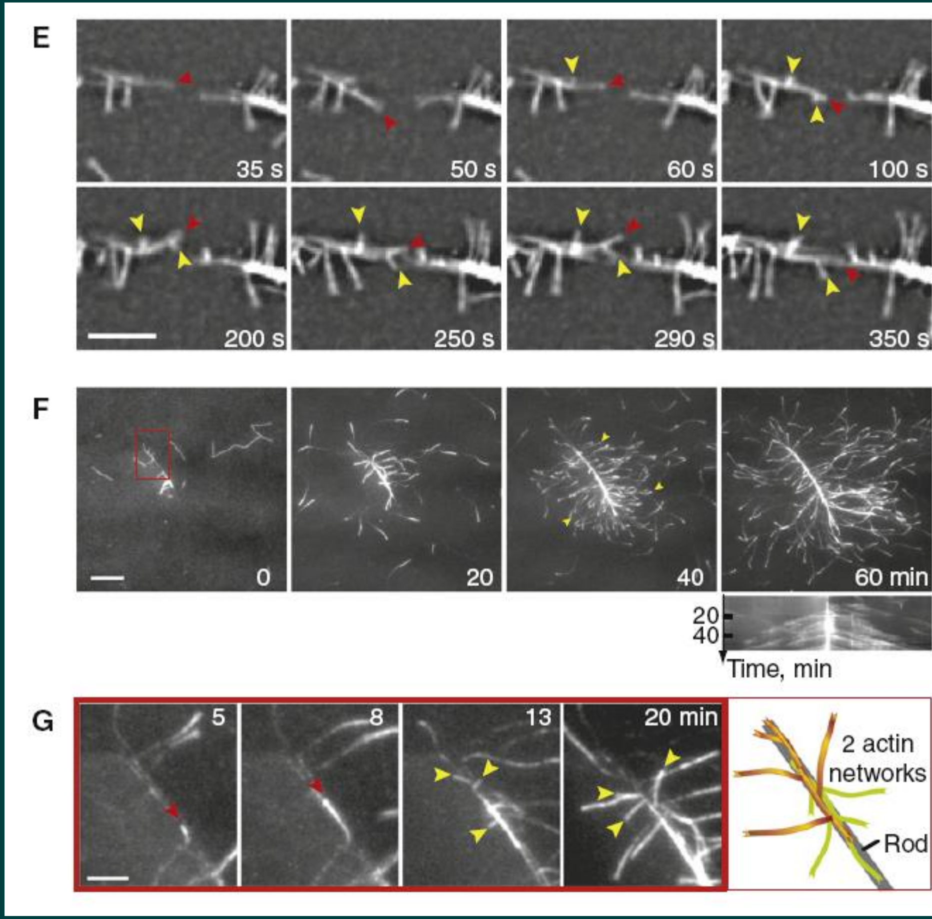
Determining filament orientation and visualizing how they push
would help to distinguish between the two models:

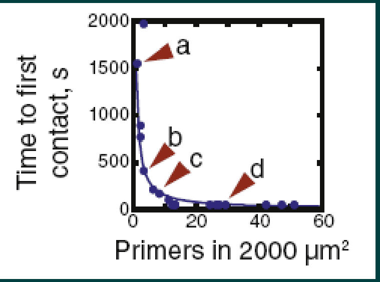
- In a reconstituted *in vitro* motility system

Achard et al., Current Biology 20, 423-28, 2010

Autoctalitic branching starts after filament "primer" contacts a particle functionalized with Arp2,3 activator. Filaments grow **away** from the particle.

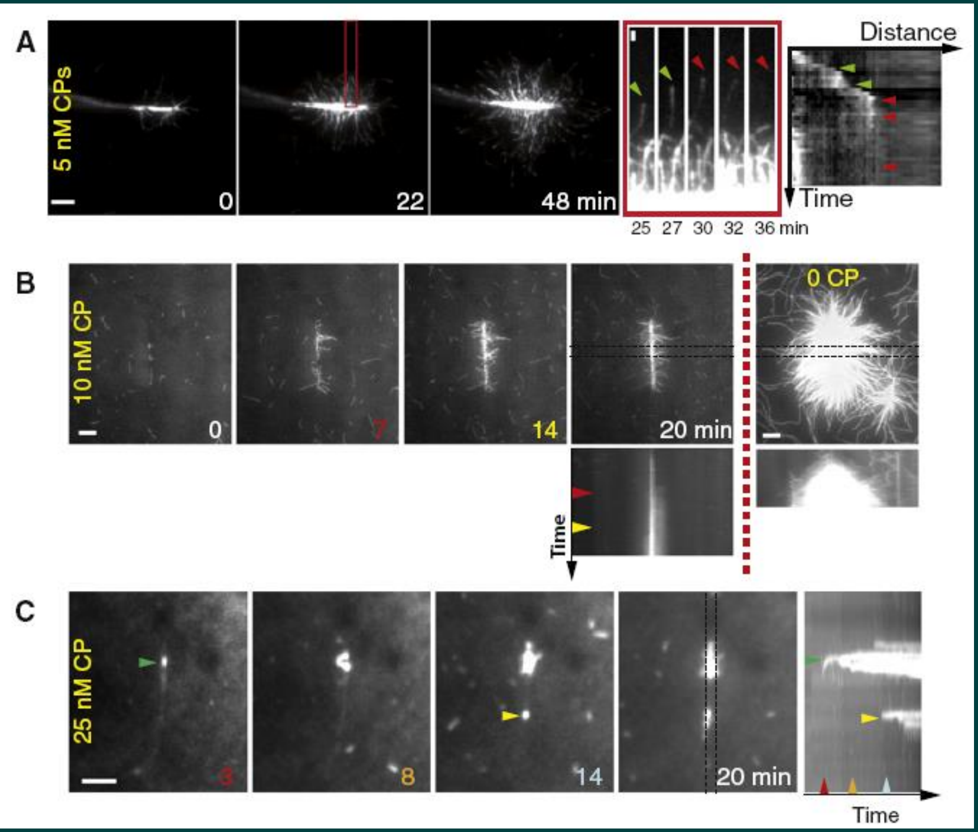




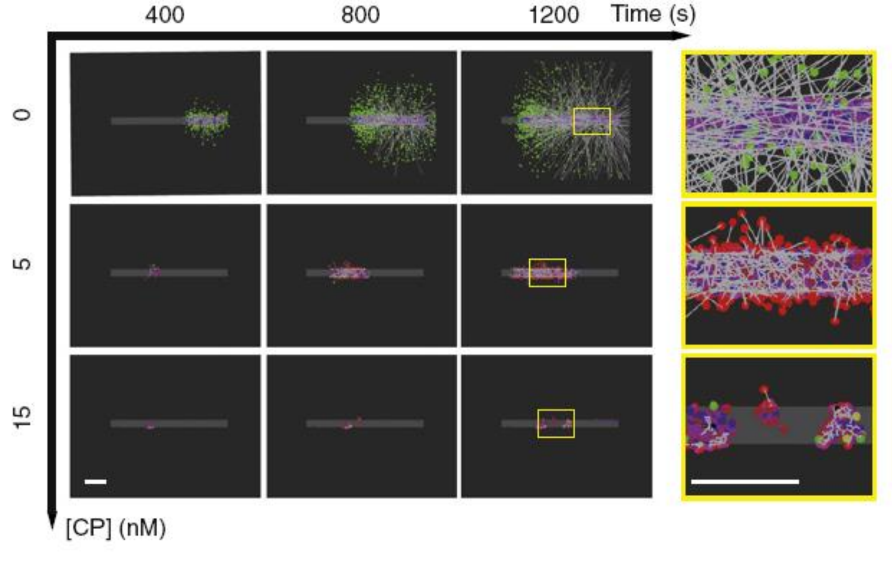


Capping protein limits the size of actin network around the particle and increases its density

experiment

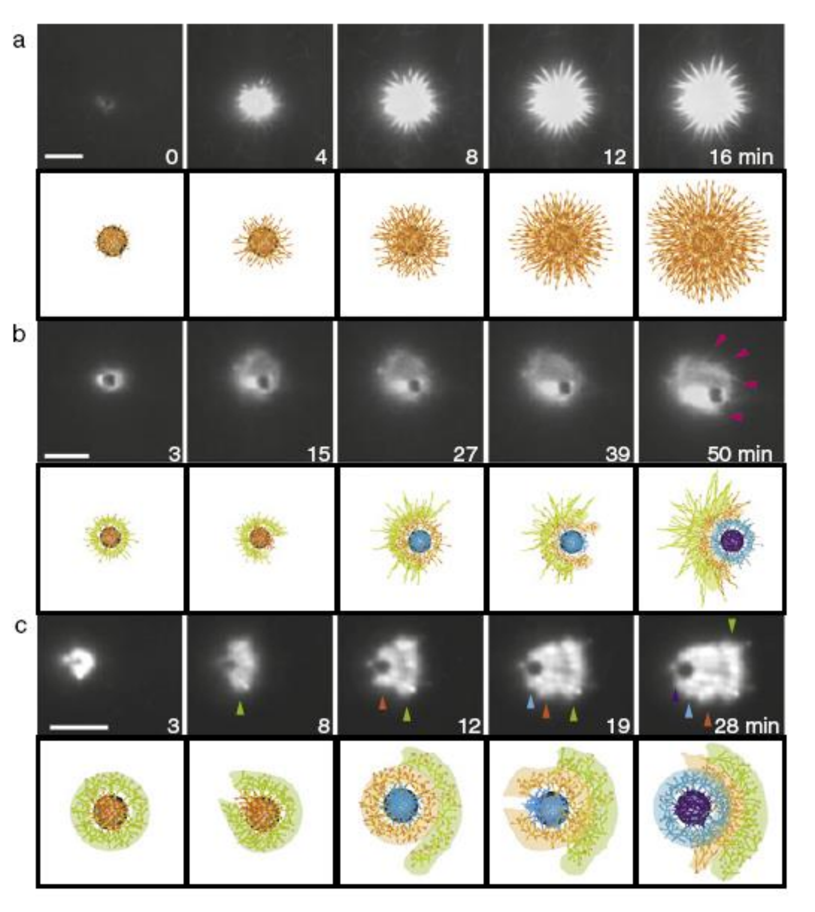


simulation

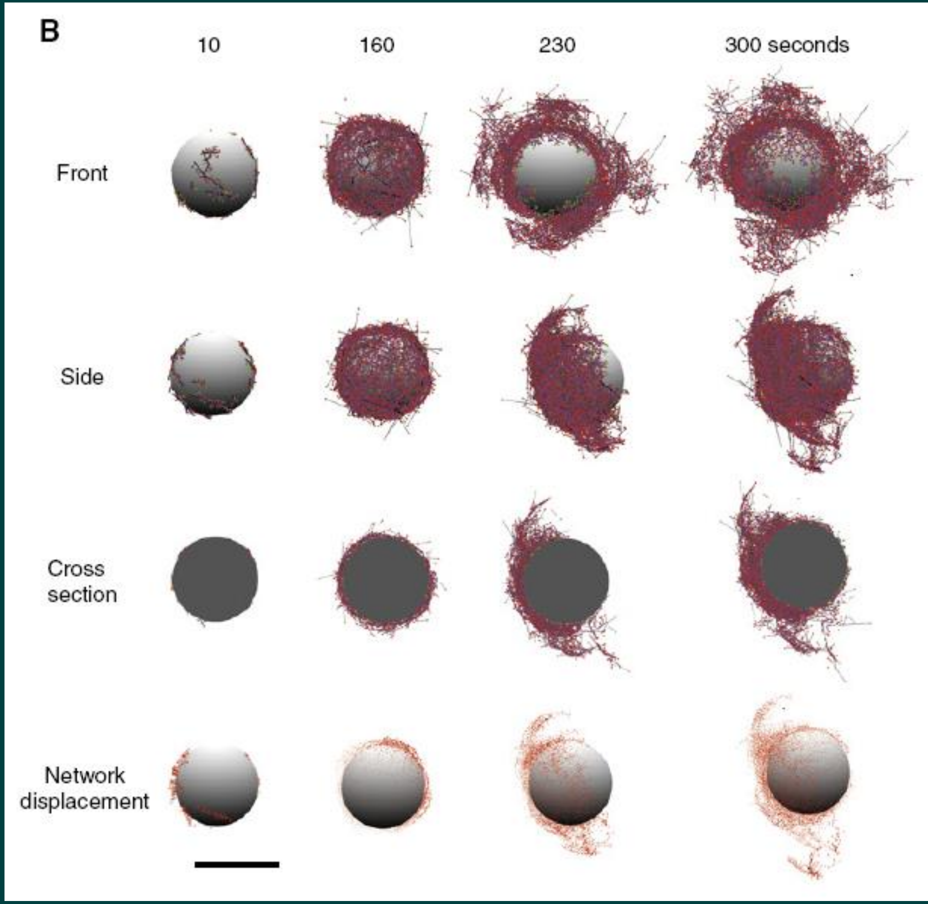


Multiple actin shell-breaking leads to motility

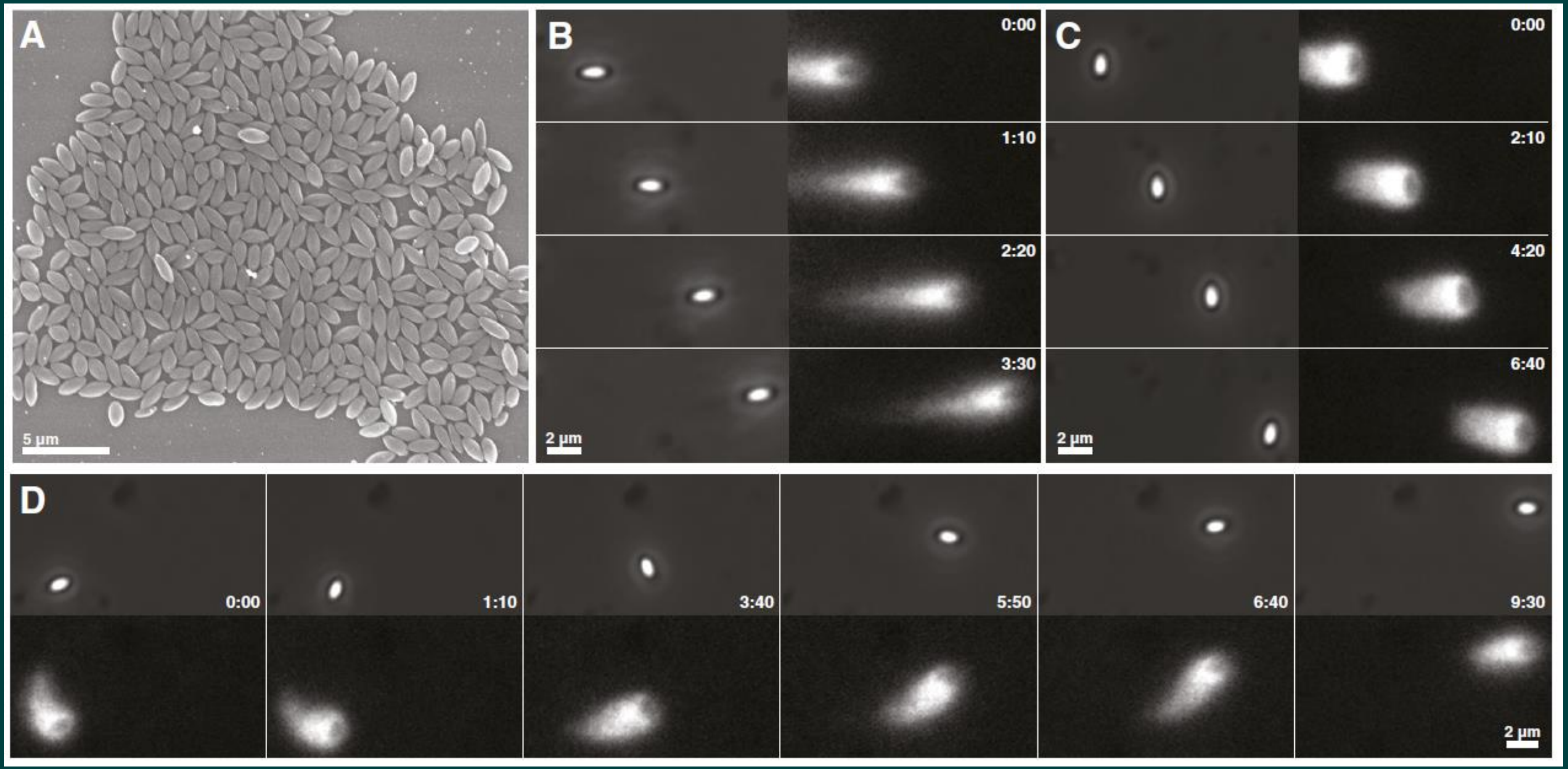
experiment



model



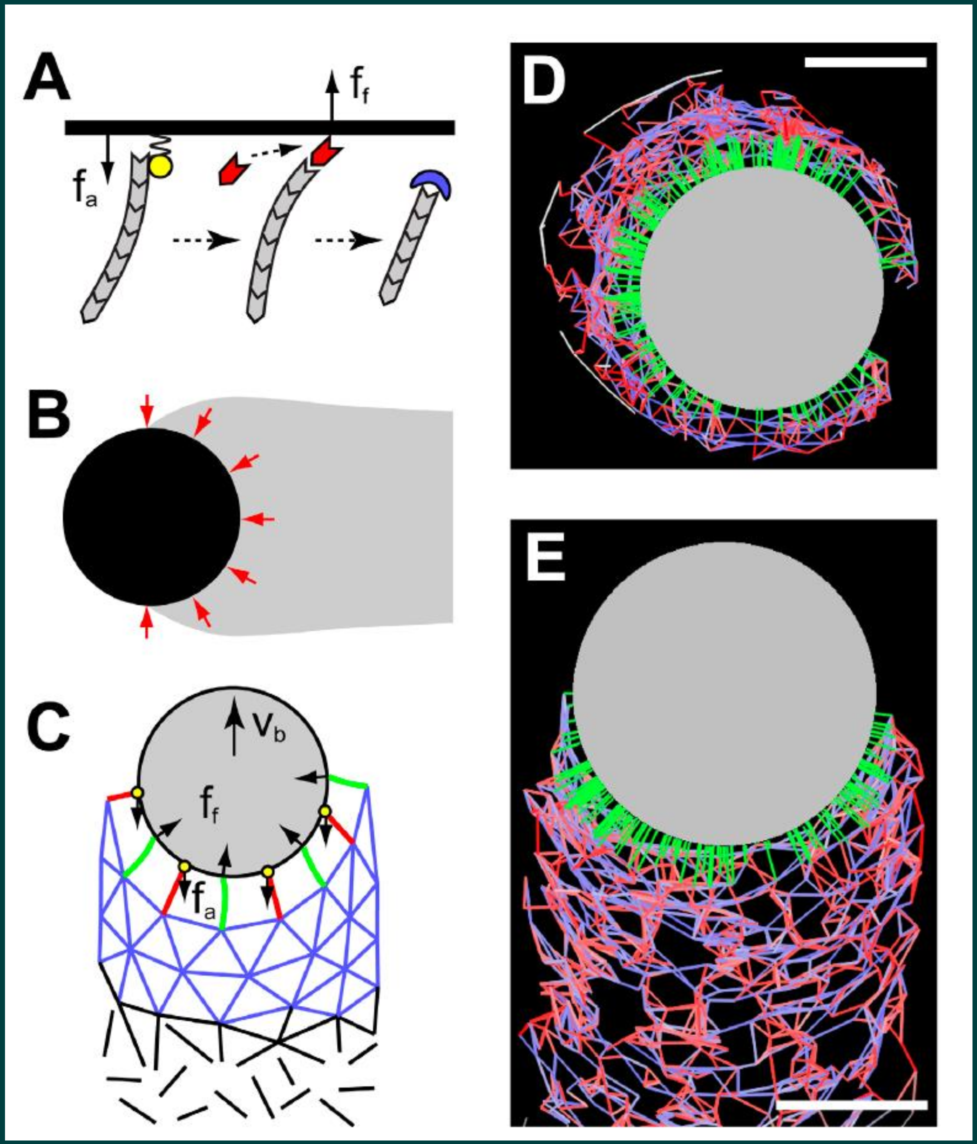
Synthes of elastic gel and Brownian ratchet models

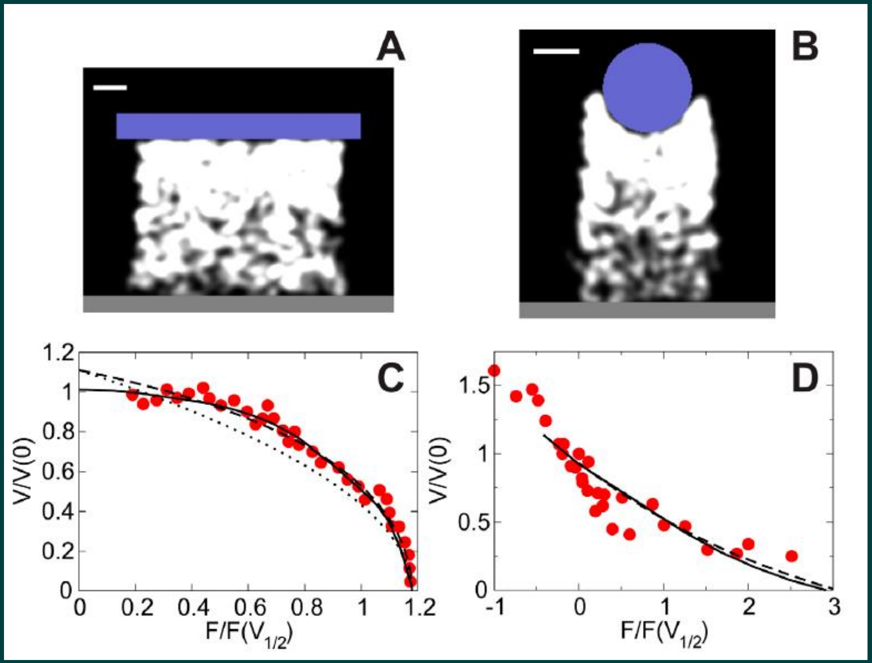


ellipsoidal beads move in two orientations

Lacayo et al., MBoC, 2012

Explained by a hybrid model





Zhu, Mogilner, PLoS Comp. Biol.

actin organization and polymerization forces in the cell:

Measuring protrusive forces in vivo

Is the filament organization at the cell edge really similar to the branching network generated in vitro?

What is the filament length?

How are filaments oriented?

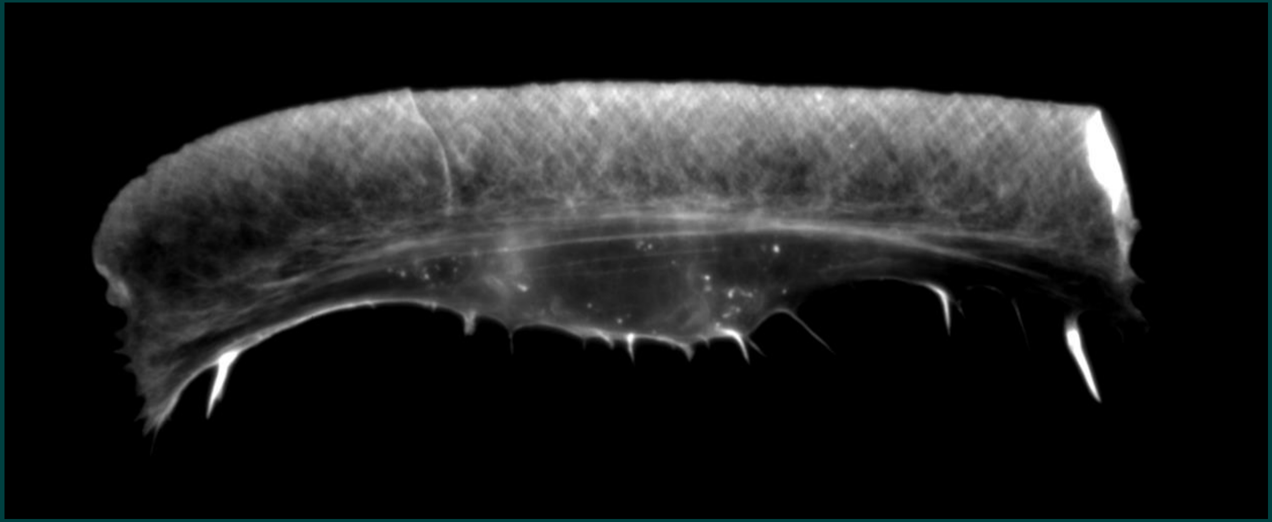
Problems of EM: difficult to measure, prone to artifacts

Brownian ratchet model implies
that filaments grow with their ends to the surface

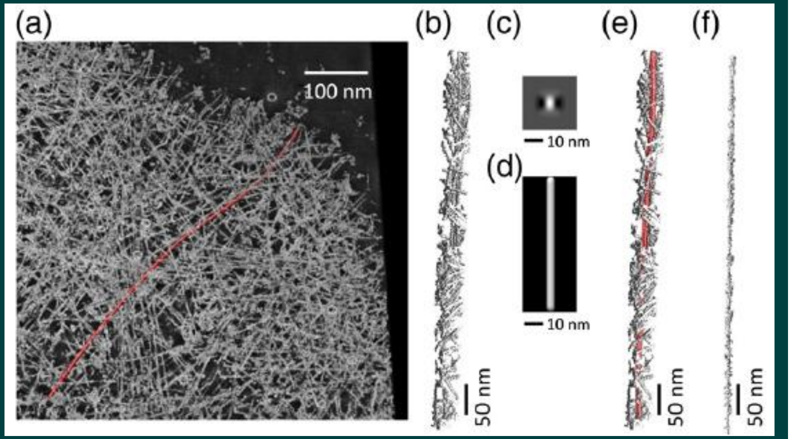
In elastic gel theory filament orientation is not important

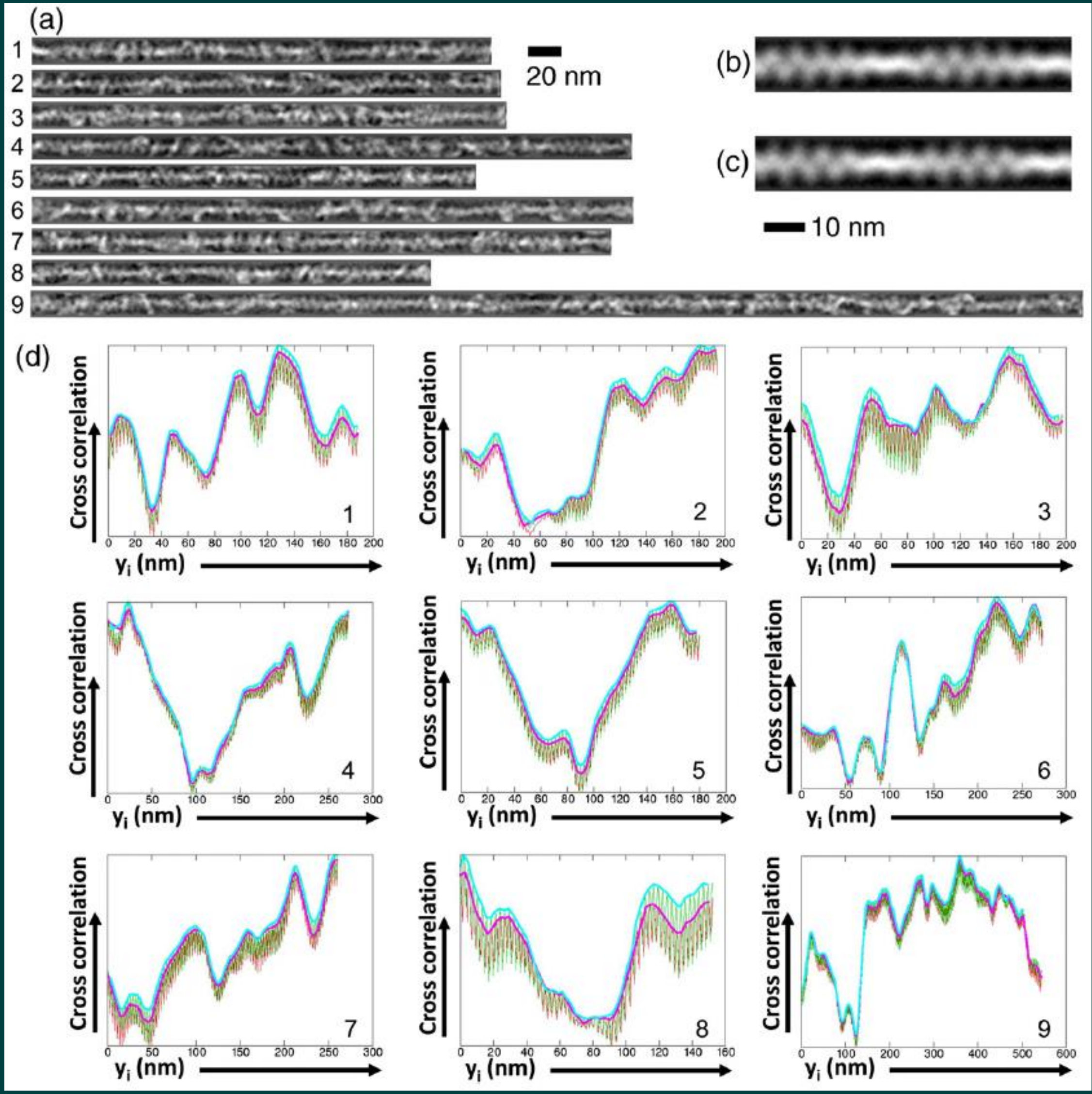
What is the filament orientation in reality?

- In the lamellipodia of migrating cells filaments are oriented with barbed ends towards the edge, consistent with Brownian ratchet model



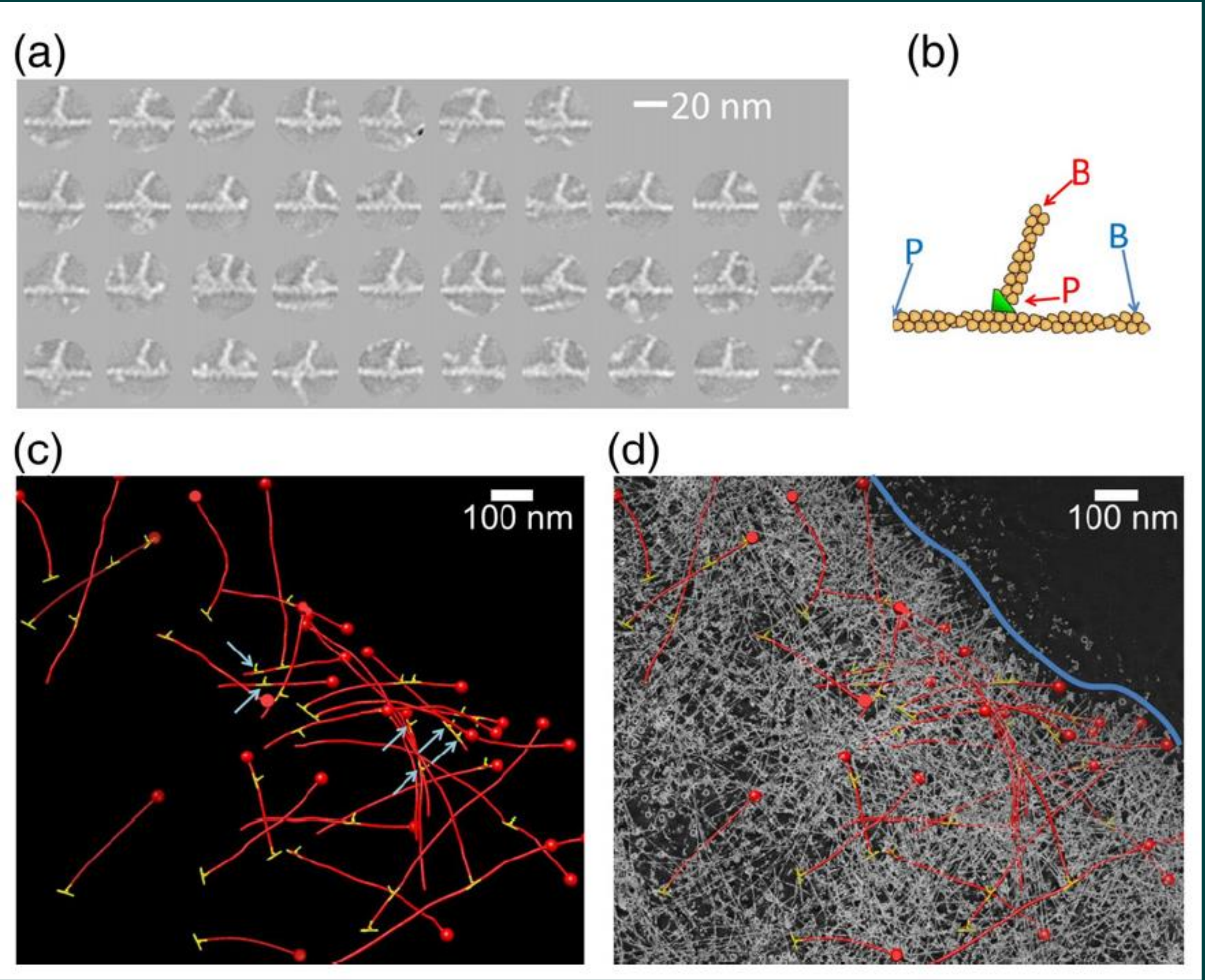
Direct determination of actin filament polarity



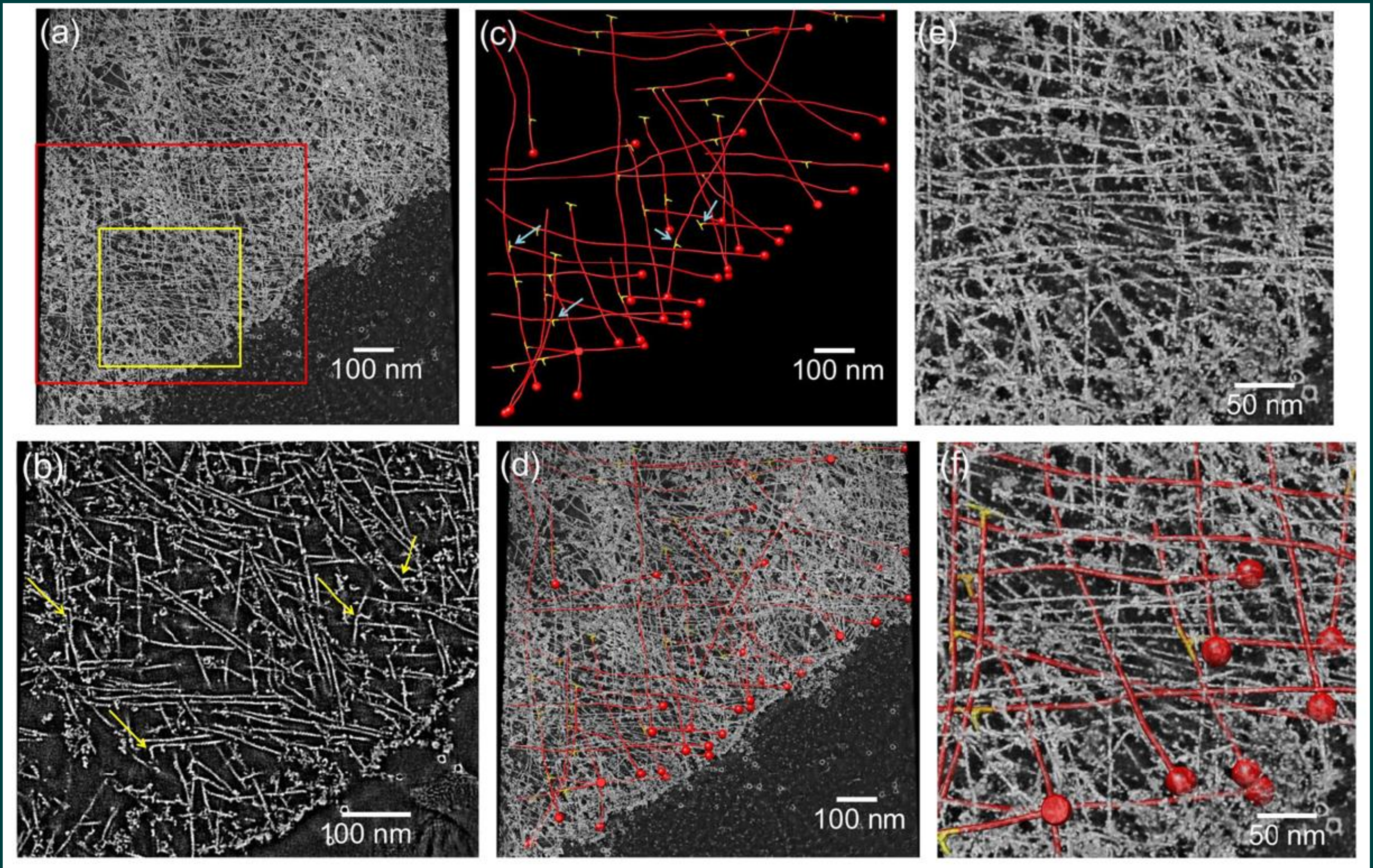


Narita et al., JMB, 2012

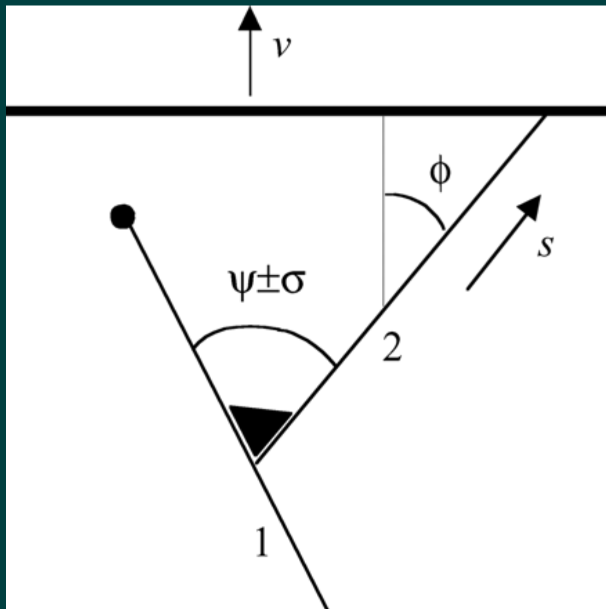
Branches and polarity

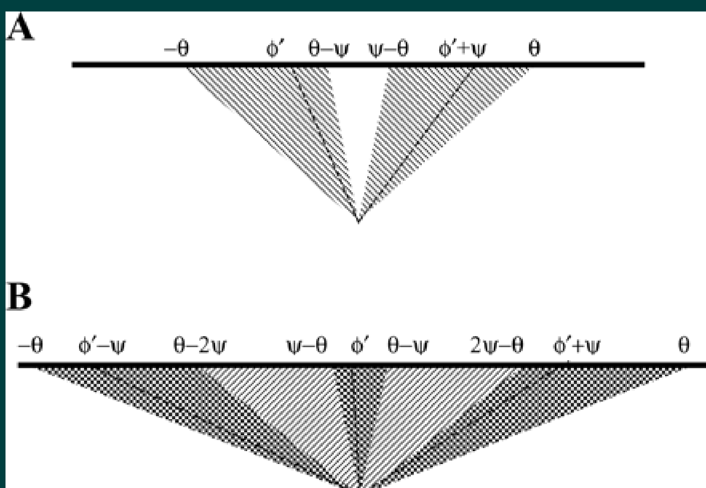


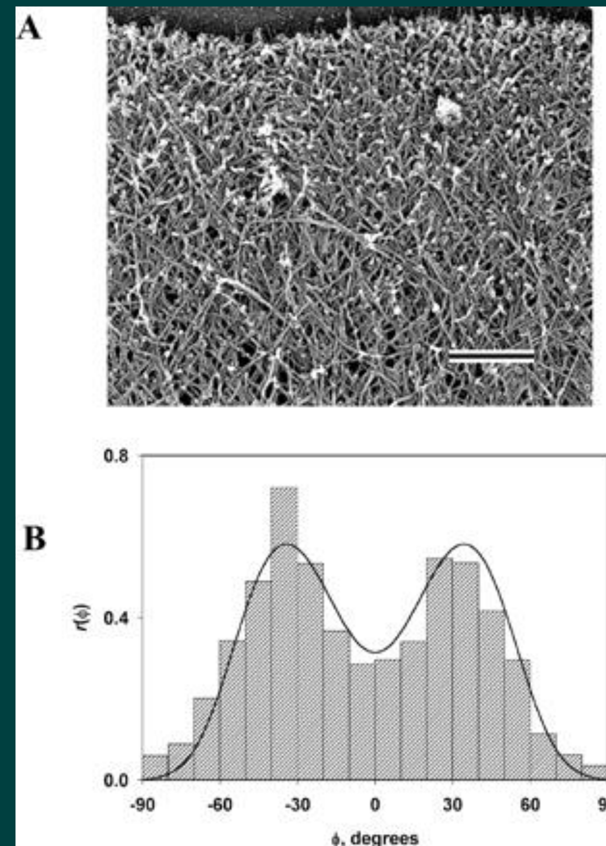
Narita et al., JMB, 2012


(a)
100 nm
(c)
100 nm
(e)
50 nm
(b)
100 nm
(d)
100 nm
(f)
50 nm

Selection of filament orientation







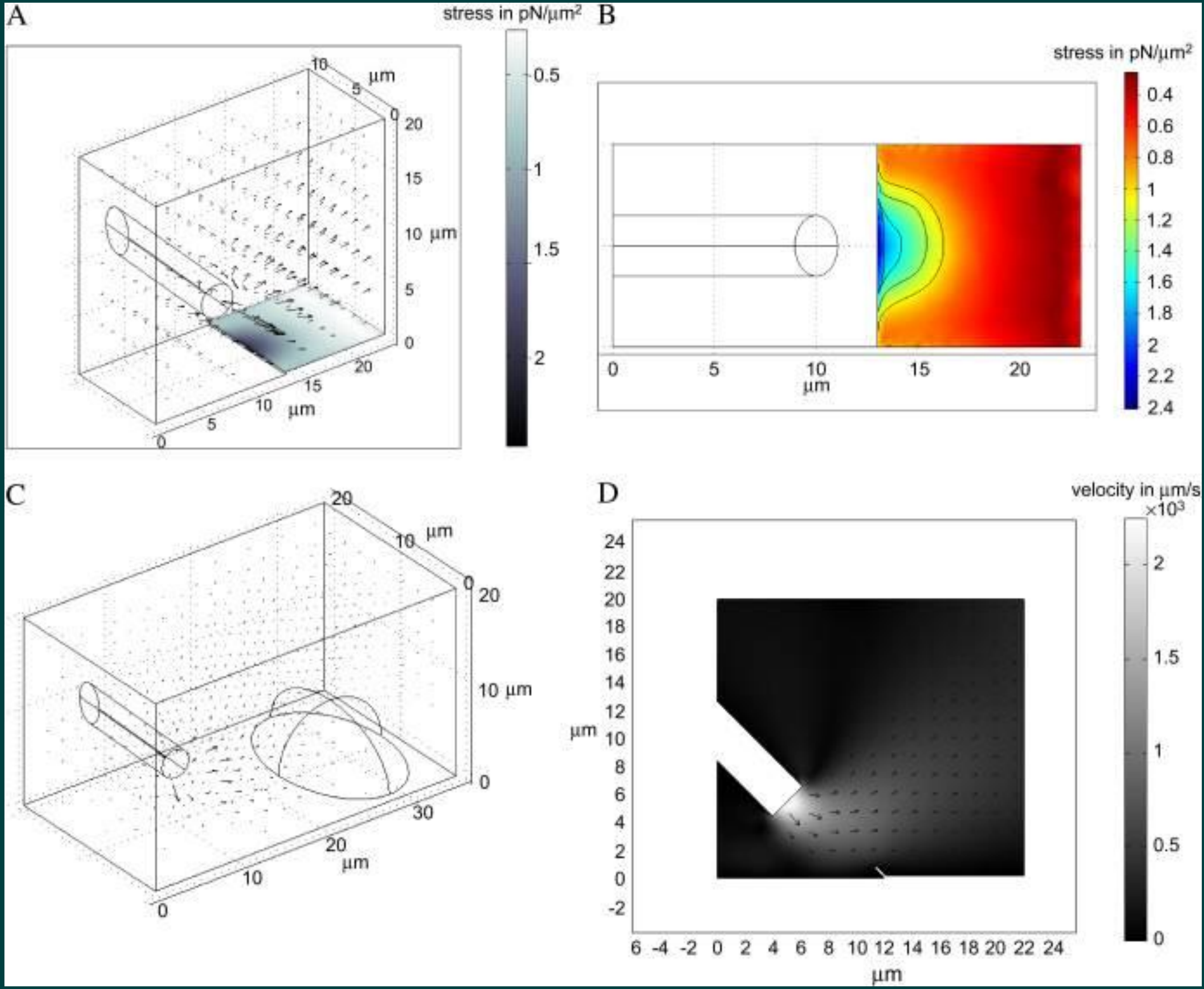
Maly IV, Borisy GG. Proc Natl Acad Sci U S A. 2001 98:11324-9

Experimental measurement of polymerization force in the cells

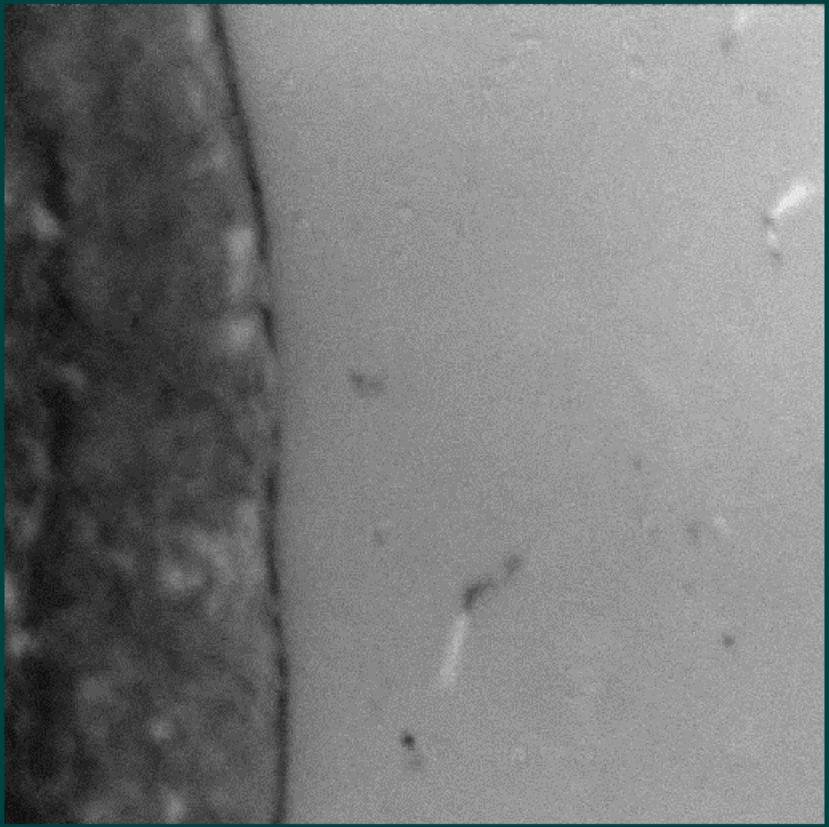
lamellipodial protrusion stalled with fluid flow

Bohnet et al., Biophys. J., 2006

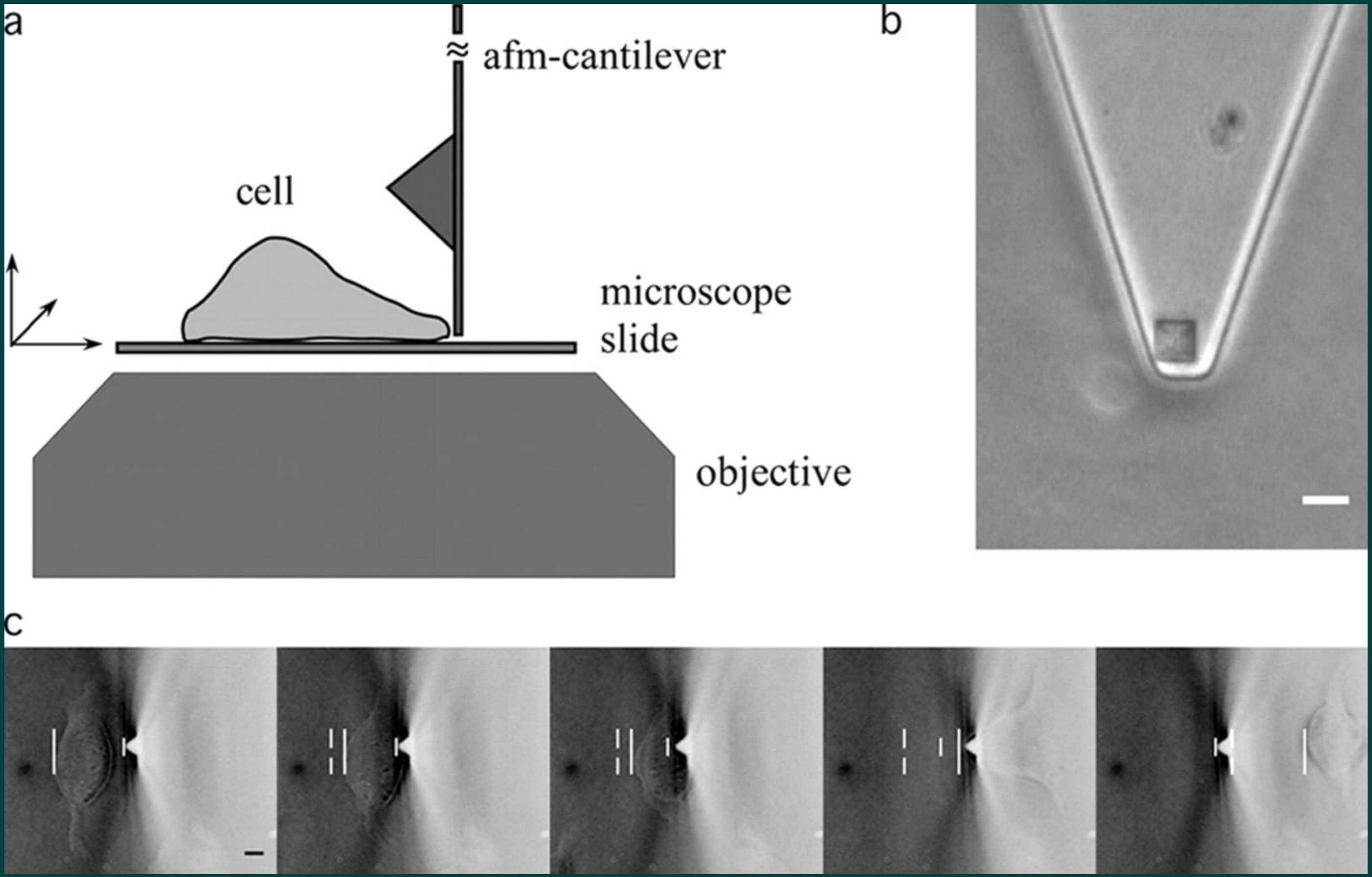
force to stall the edge estimated in a few pN range



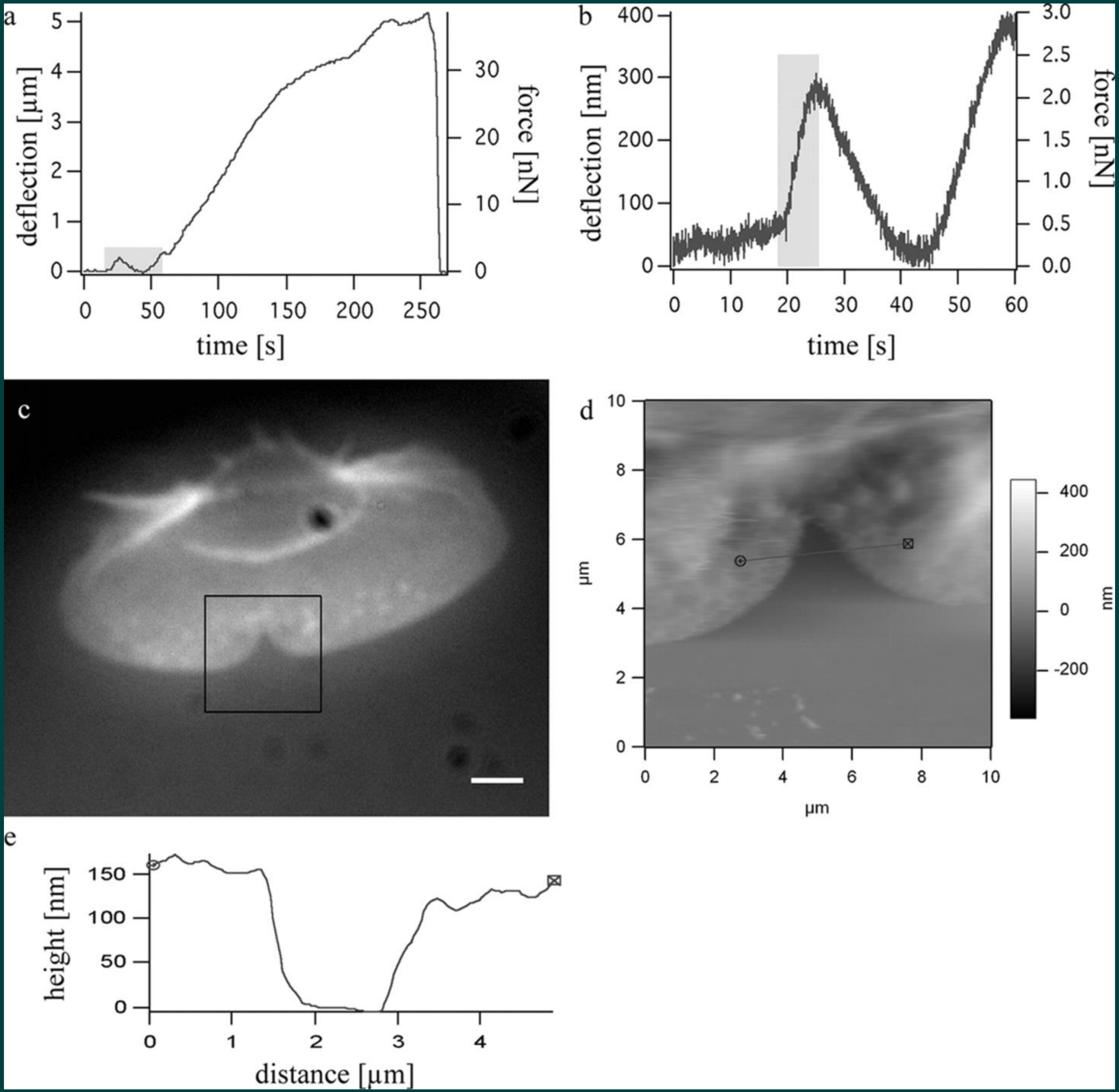
IRM: competition between the external force and the nascent contacts at the very tip of the lamellipodia



Measuring protrusive force with the AFM

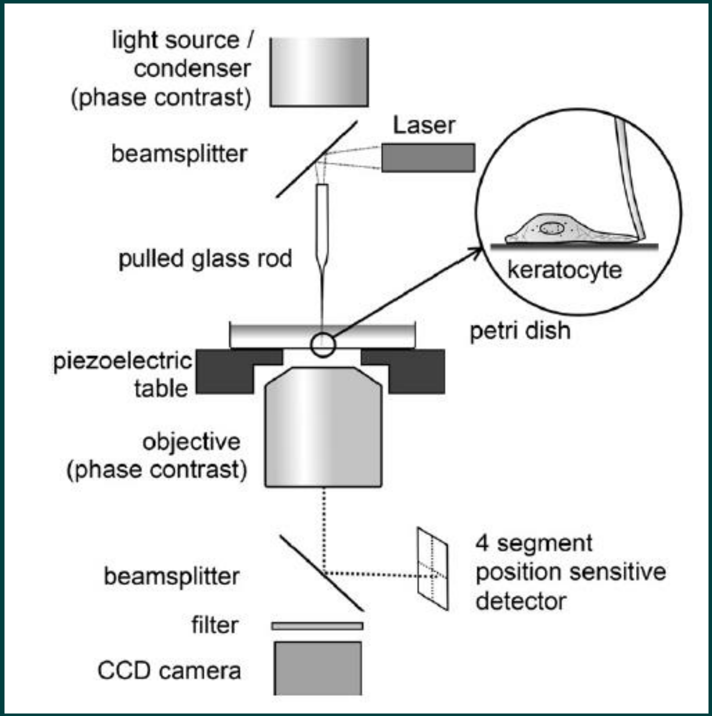


Prass et al., J. Cell Biol., 2006

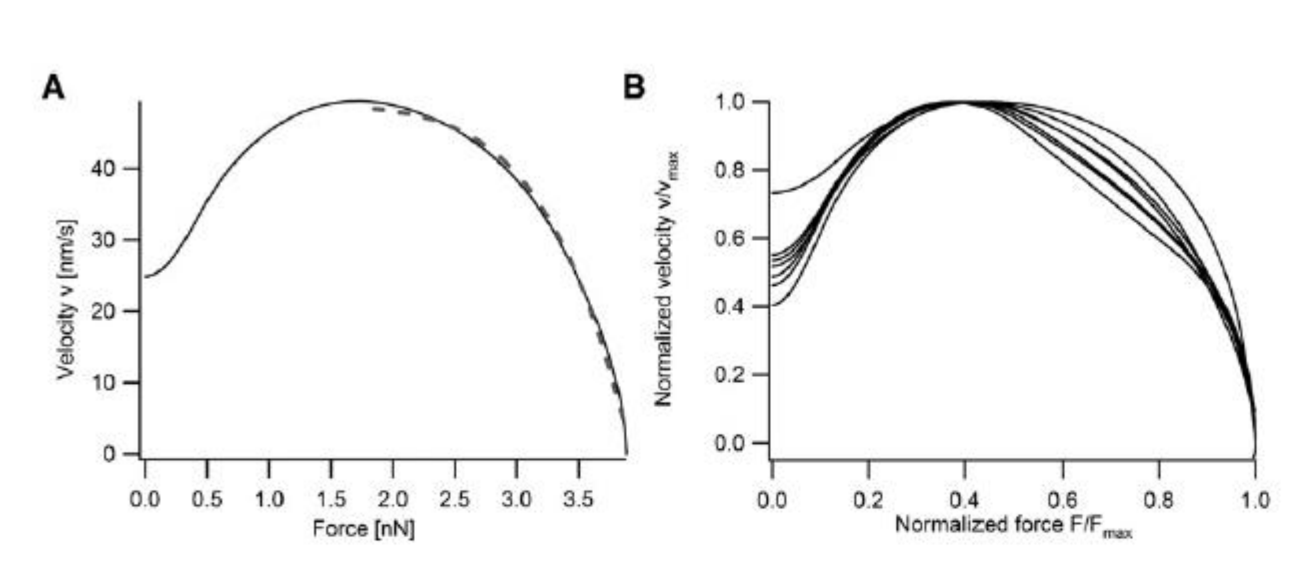


a
deflection [µm]
force [nN]
time [s]
b
deflection [nm]
force [nN]
time [s]
c
d
µm
nm
e
height [nm]
distance [µm]

With optical fiber



Heinemann et al., BJ, 2011



Filament reorganization and/or deformation may be responsible for convex up force-velocity profiles in vivo

modes of filament deformation

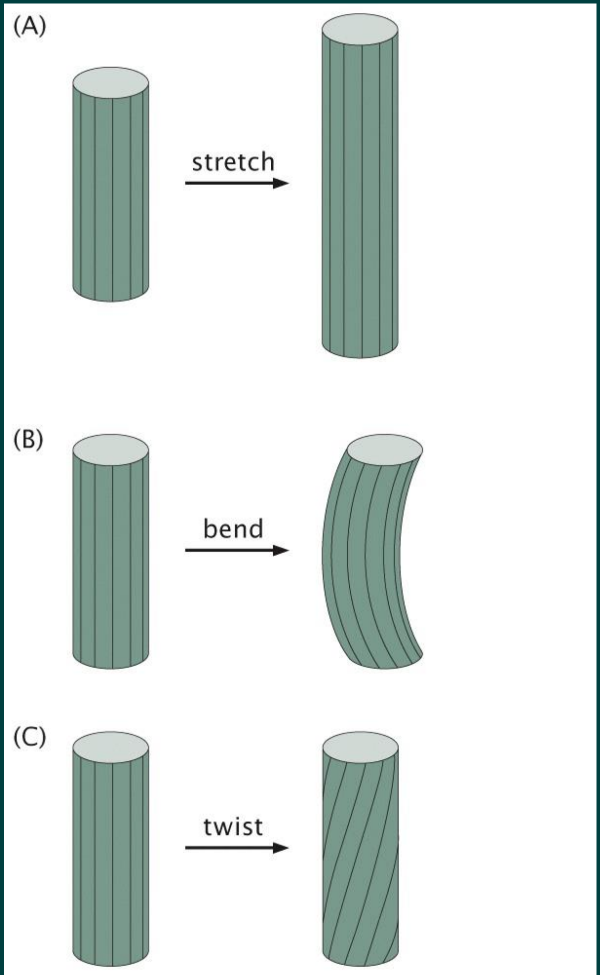


Figure 10.2 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

filaments are flexible
persistence length

Figure 10.30 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

$$k_B T \approx \frac{EIL}{2R^2}$$

for L = R = persistence length

$$\xi_P \approx \frac{EI}{2k_B T}$$

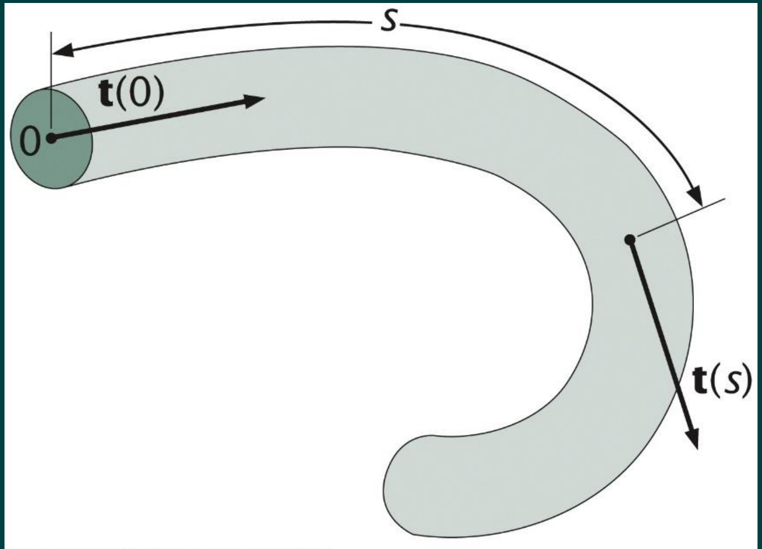


Figure 10.7 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

from correlation of tangent vectors

$$g(s) = \langle \mathrm{t}(s) \cdot \mathrm{t}(0) \rangle$$

$$\xi_P = \frac{EI}{k_B T}$$

$$g(s) = e^{-\frac{s}{\xi_P}}$$

$$EI = \xi_P k_B T$$

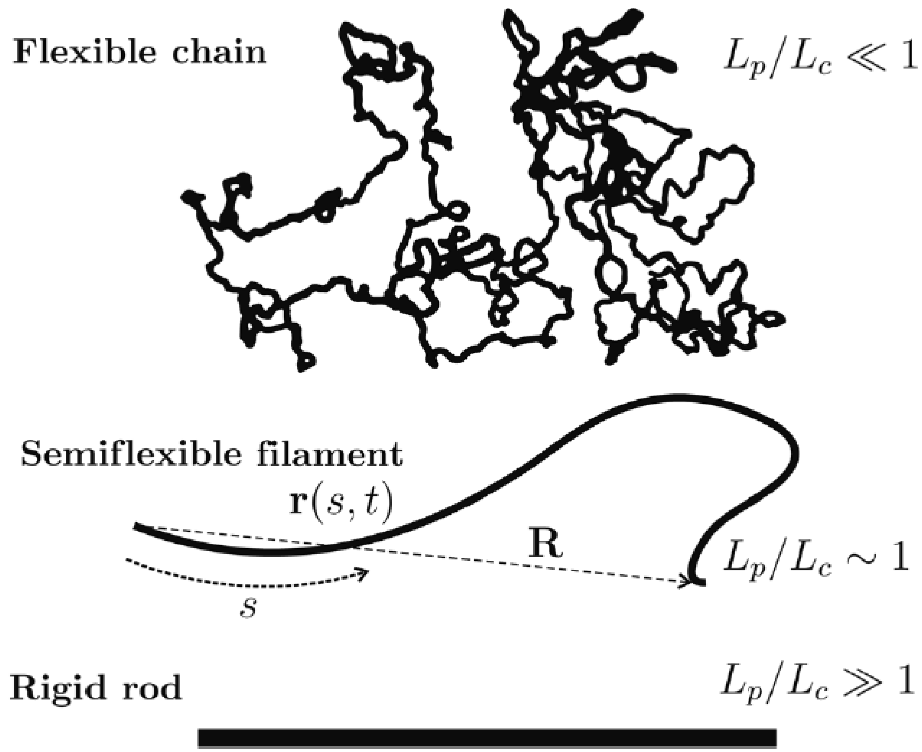
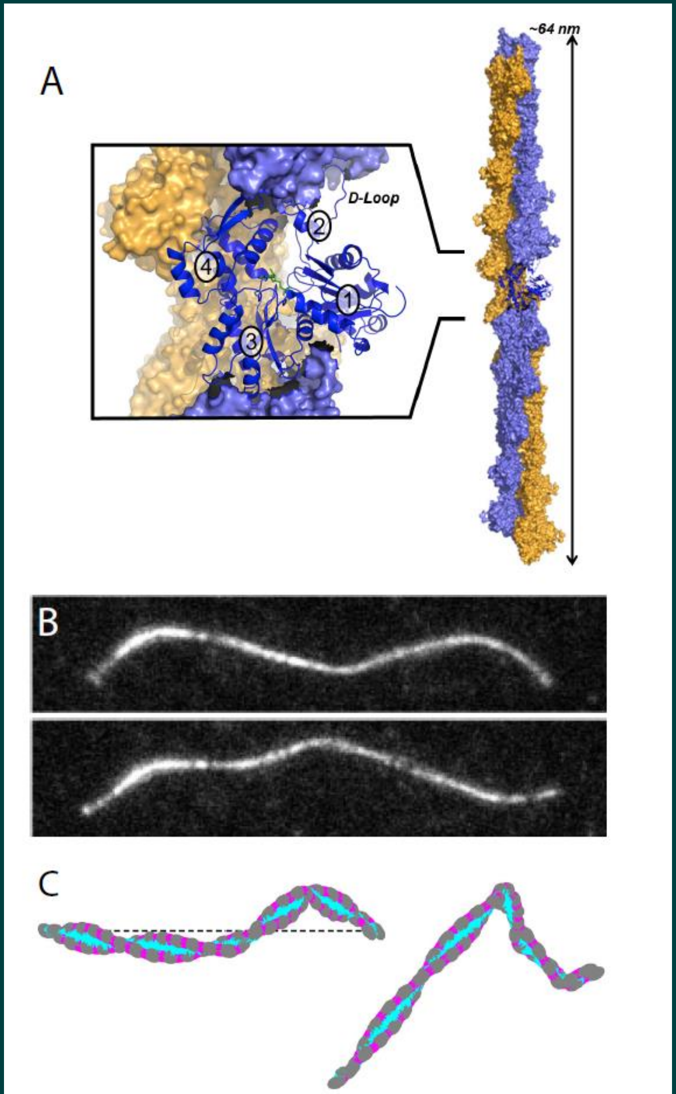


Fig. 1 Chain conformation depends on persistence length, L_p, and contour length, L_c. Flexible chains take a random coil formation, semiflexible filaments are comparatively straight with thermally induced bending undulations, and rigid rods are not influenced by thermal energy.

Persistence length L_p

Pritchard et al., Soft Matter, 2014

Actin filaments: bending, torsional and coupled twist-bend persistence lengths

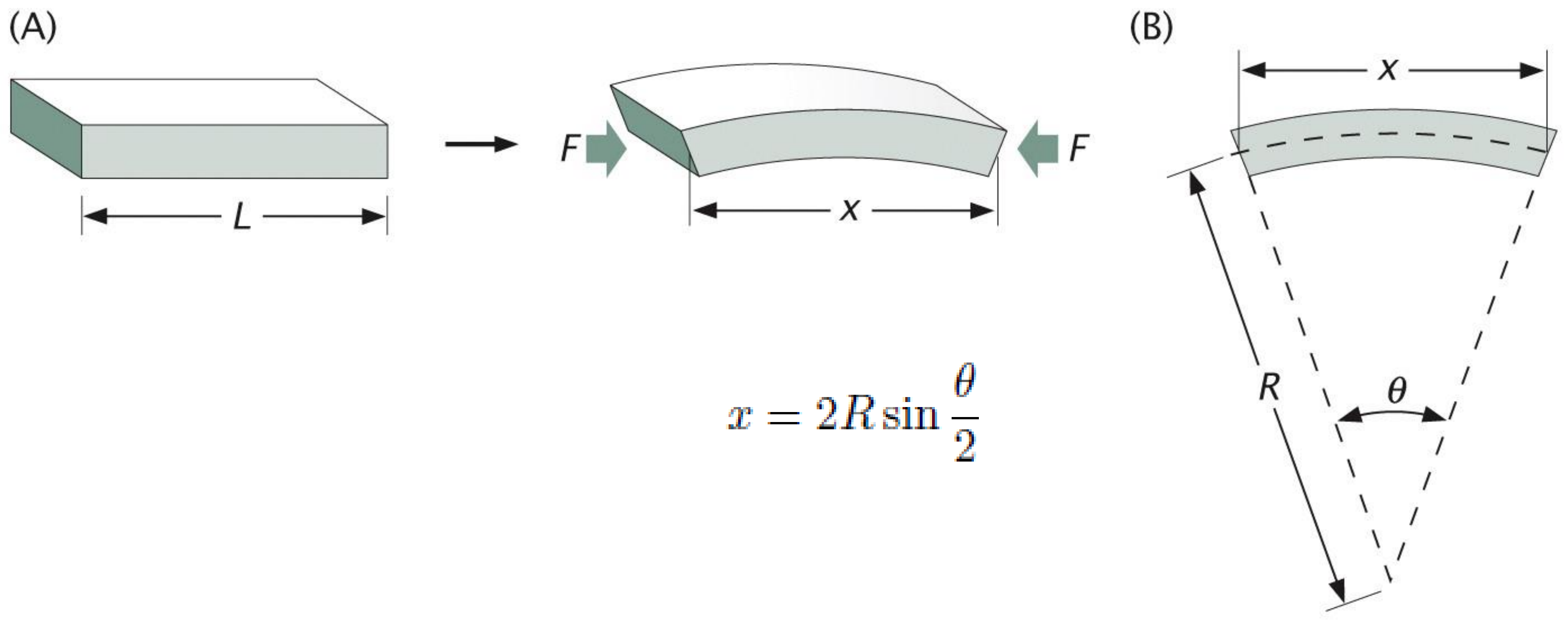


D

	L_B	L_T	L_{TB}
ATP	13 µm	1.4 µm	0.4 µm
ADP-Pi	10 µm		
ADP	9.5 µm	0.5 µm	0.2 µm

De la Cruz and Gardel, 2015

to buckle, work of buckling force should exceed strain energy of bending



$$x = 2R\sin\frac{\theta}{2}$$

Figure 10.33 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

$$EI = \xi_P k_B T$$

$$E_{\text{tot}} = \frac{\xi_P k_B T}{2}\frac{L}{R^2} - F(L - x)$$

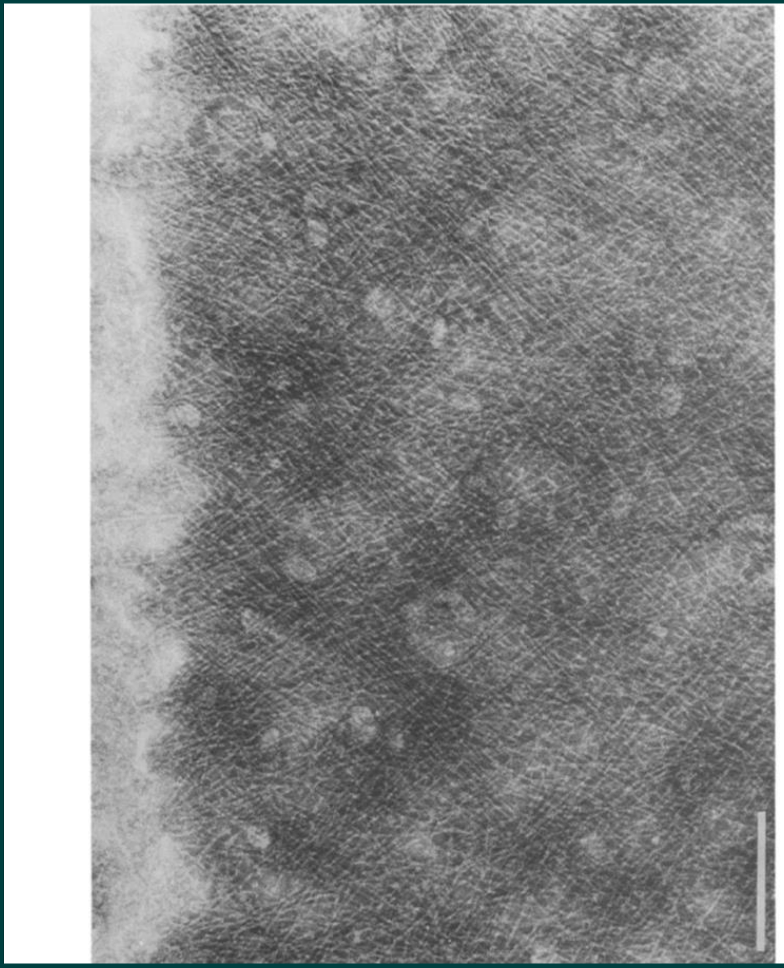
$$F_{\text{crit}} = 12\frac{k_B T \xi_P}{L^2}$$

$$\frac{E_{\text{tot}}}{k_B T} = \frac{\xi_P}{L}\frac{\theta^2}{2} - \frac{FL}{k_B T}\left(1 - \frac{2}{\theta}\sin\frac{\theta}{2}\right)$$

for 1 µm actin filament with ξ = 10 µm

F_{crit} = 0.5 pN

long filaments or short filaments?



Small, Herzog and Anderson, 95

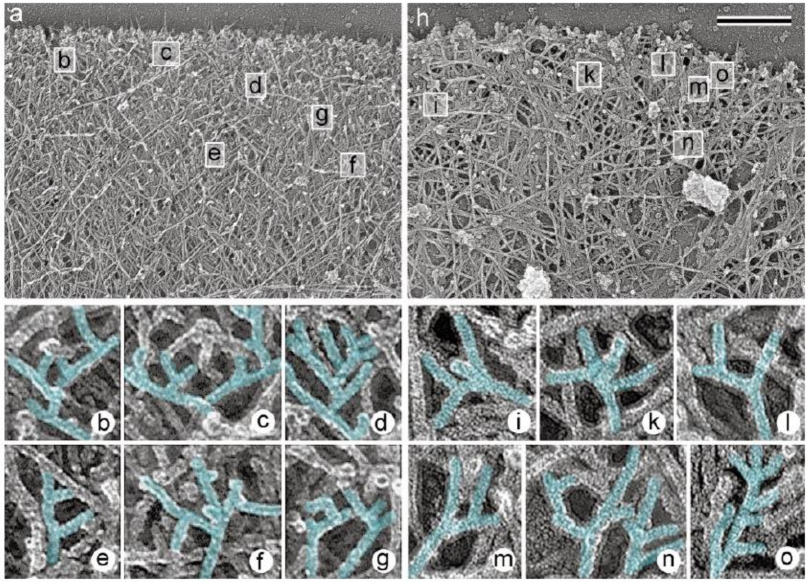


Figure 1. Multiple branching of actin filaments in lamellipodia. EM of lamellipodia of *Xenopus* keratocytes (a–g) and fibroblasts (h–o) showing overviews of the leading edge (a and h) and enlargements of the boxed regions (b–g and i–o). Many examples of filaments with tightly spaced multiple branches (cyan) can be visualized in lamellipodia despite the high overall density of the actin network. Bar, 0.5 μm.

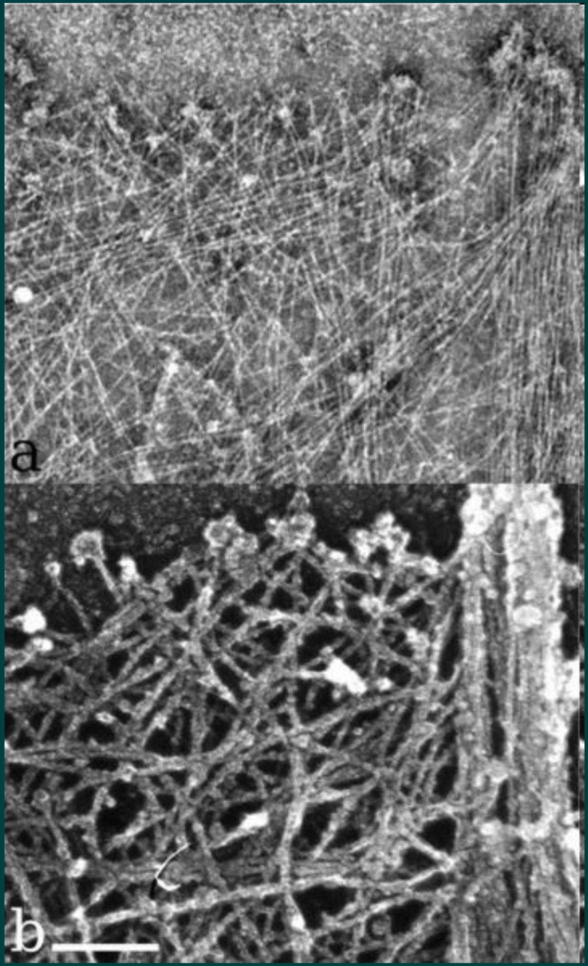
Svitkina and Borisy, 99

Related to length:
network stiffness,
branching and capping frequency

Short branching filaments – real or artifact of EM?

negative staining

electron tomography



freeze-drying/metal replication

Small et al., 2008

next time:

estimation of filament length with optical microscopy

how the cell makes long, unbranched filaments

molecular motors