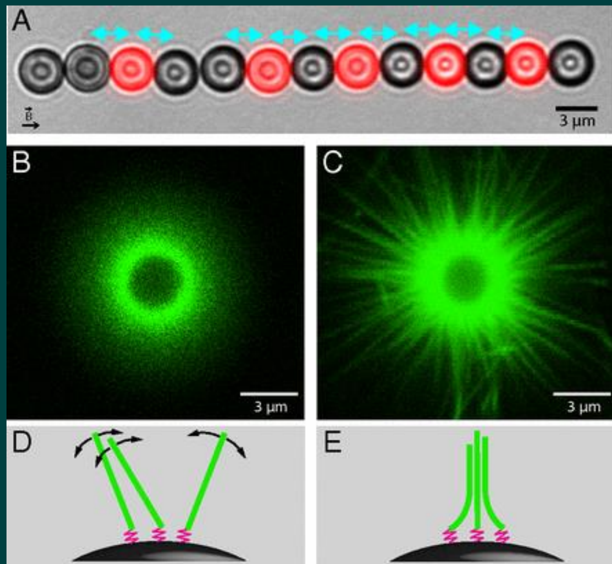
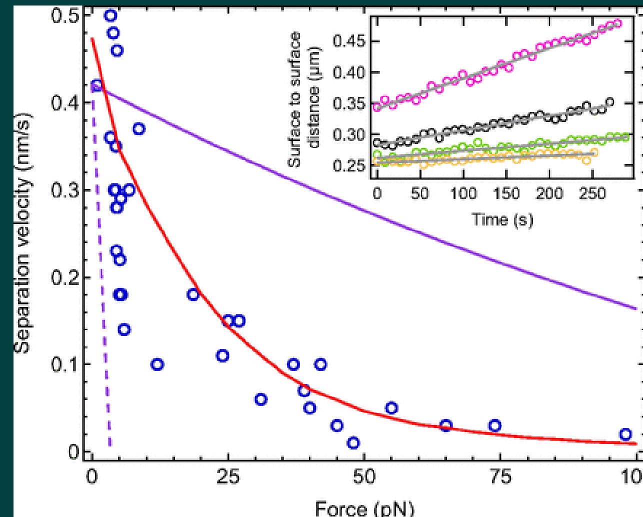
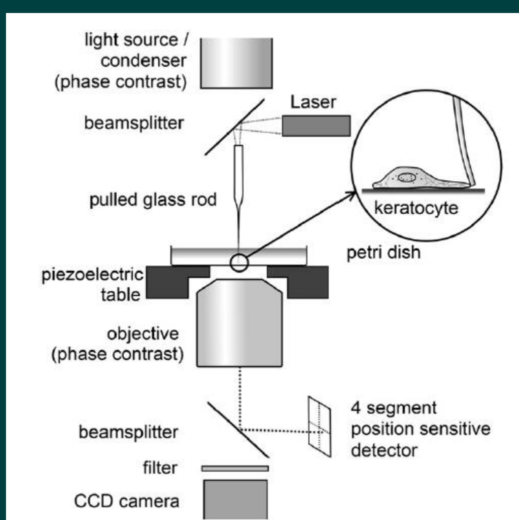
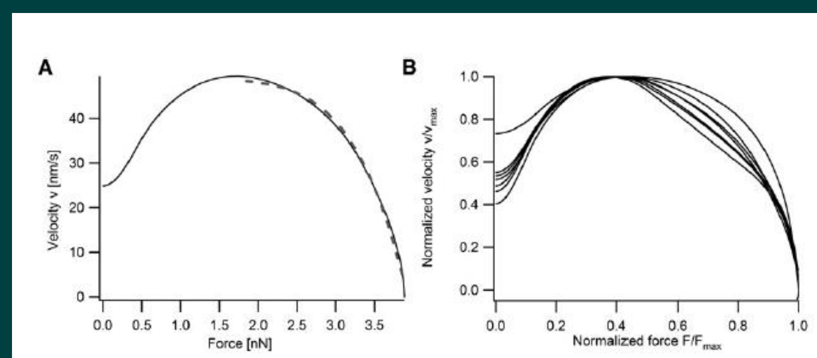
last time: Force production by actin filament assembly in vitro and in vivo





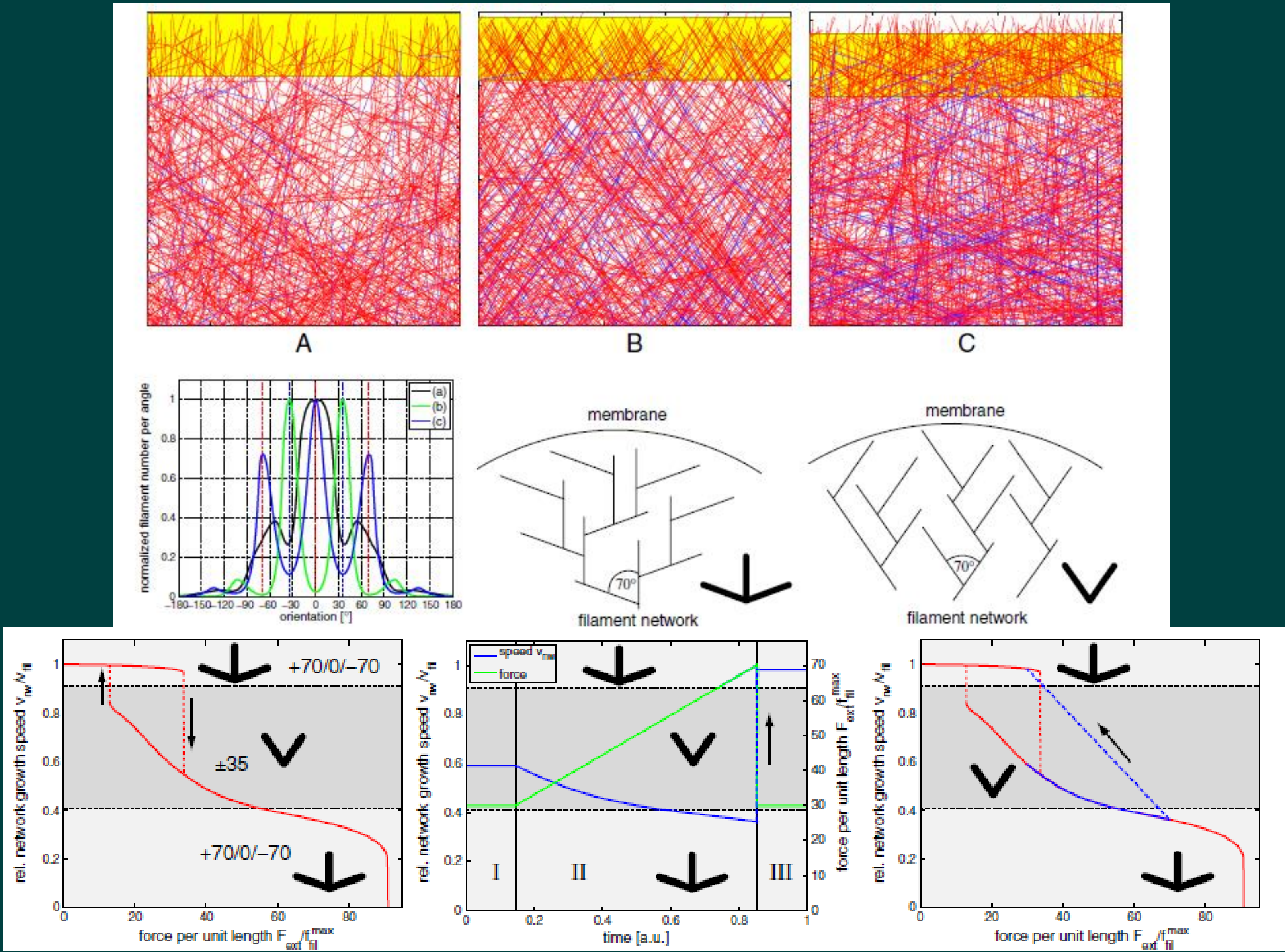
Démoulin et al, 2014





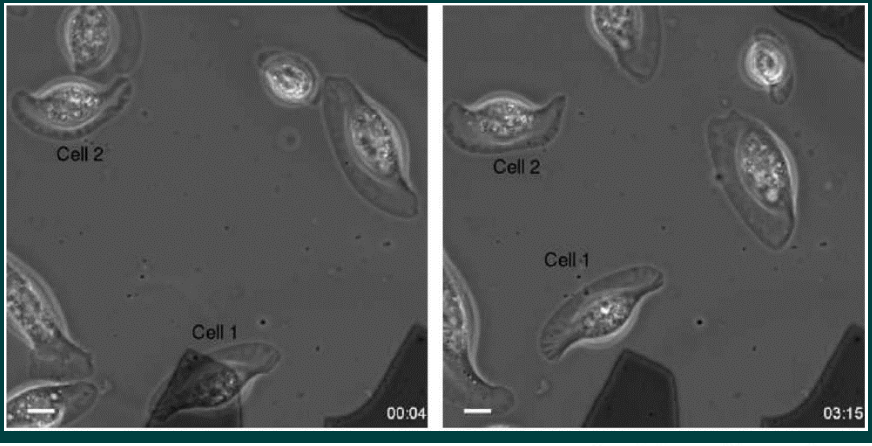
Heinemann et al., 2011

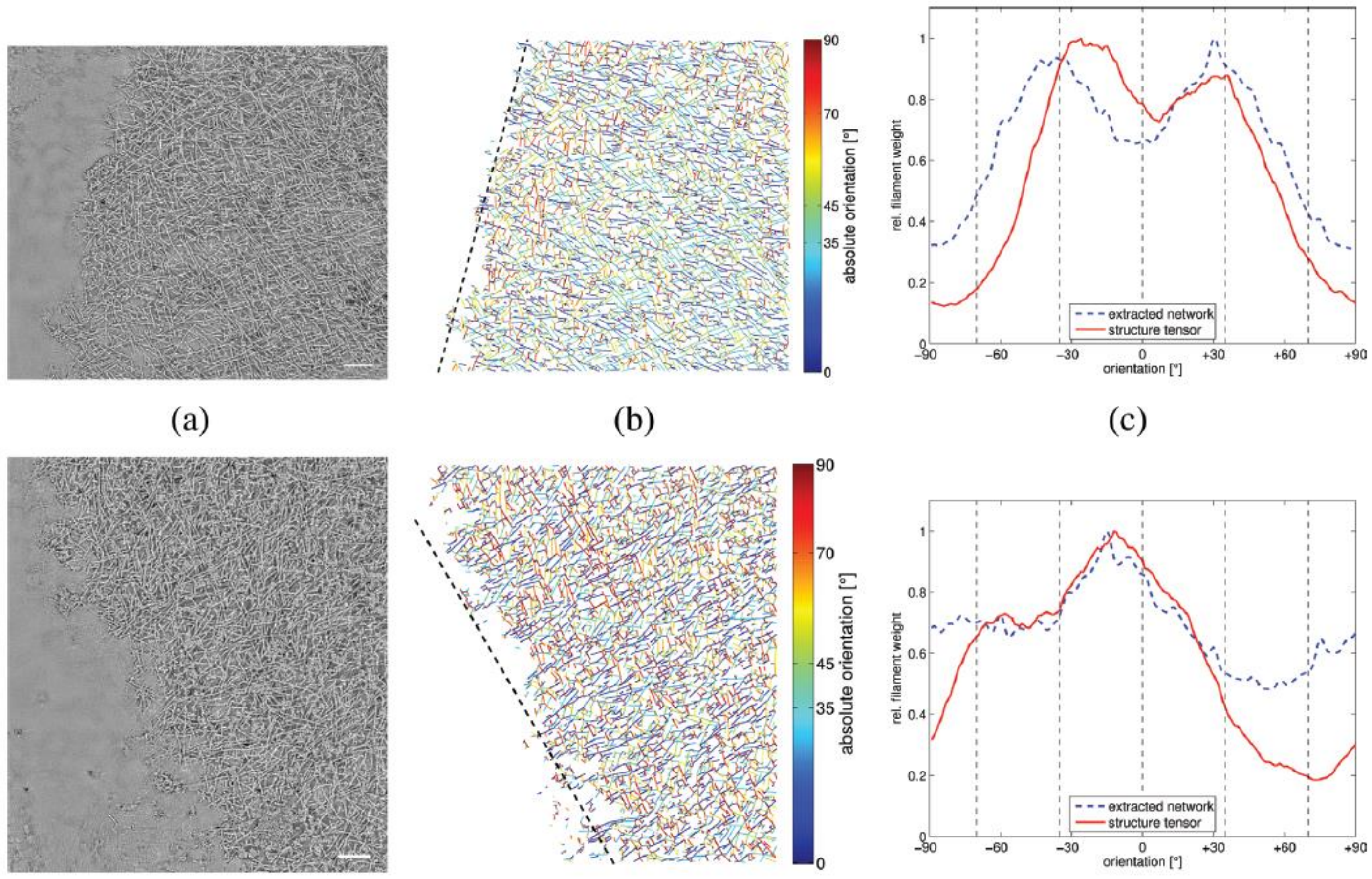
Orientation matters: Anomalies in force-velocity relationship result from change of filament orientation



Weichsel and Schwarz, PNAS, 2010

Filament orientation in fast and slow cells





Weichsel et al., 2012

Filament length matters for force production

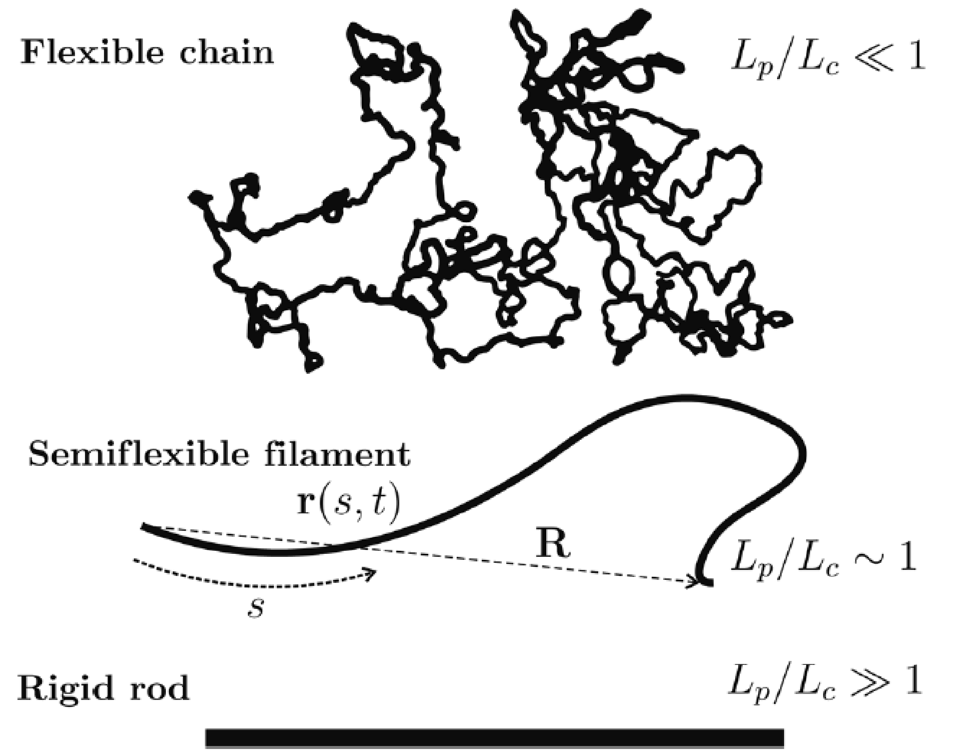


Fig. 1 Chain conformation depends on persistence length, L_p, and contour length, L_c. Flexible chains take a random coil formation, semiflexible filaments are comparatively straight with thermally induced bending undulations, and rigid rods are not influenced by thermal energy.

Persistence length L_p

buckling force

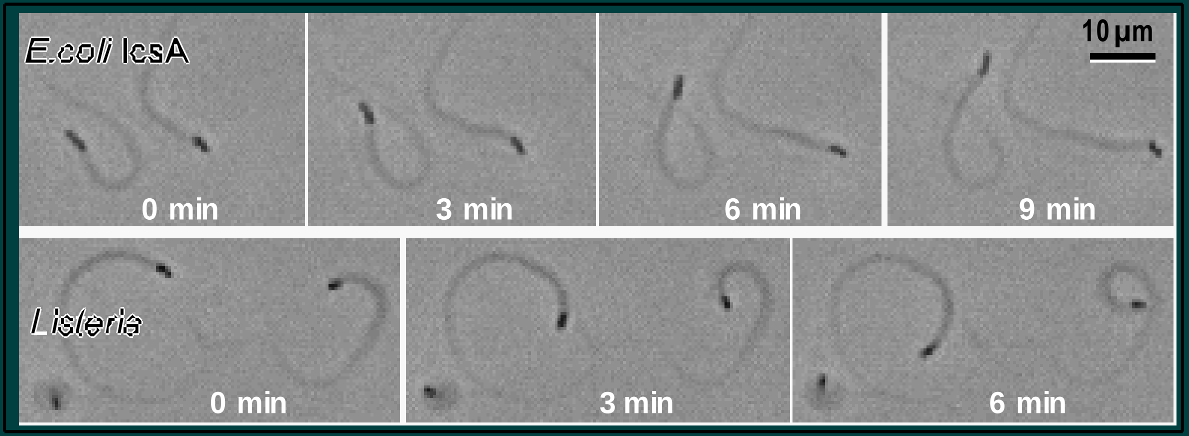
$$F_{\text{crit}} = 12\frac{k_B T \xi_P}{L^2}$$

for 1 µm actin filament with ξ = 10 µm

F_{crit} = 0.5 pN

Pritchard et al., Soft Matter, 2014

What proteins could regulate actin filament length in the branching network?



Rate :
2 to 3 µm/min

Essential Proteins :

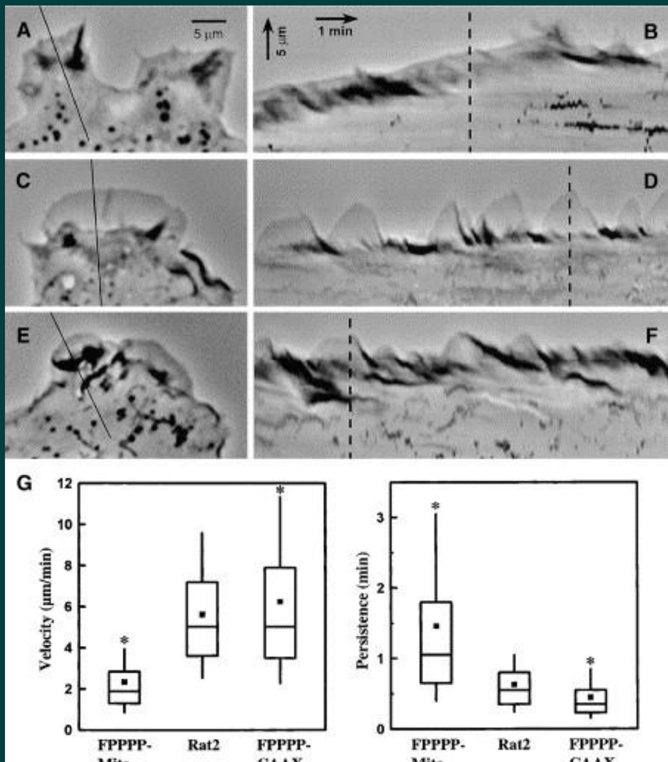
N-WASP	IcsA-bound
Arp2/3	0.1 µM
Capping Protein	0.1 µM
ADF	2 µM
ATP-actin+F-actin	8 µM

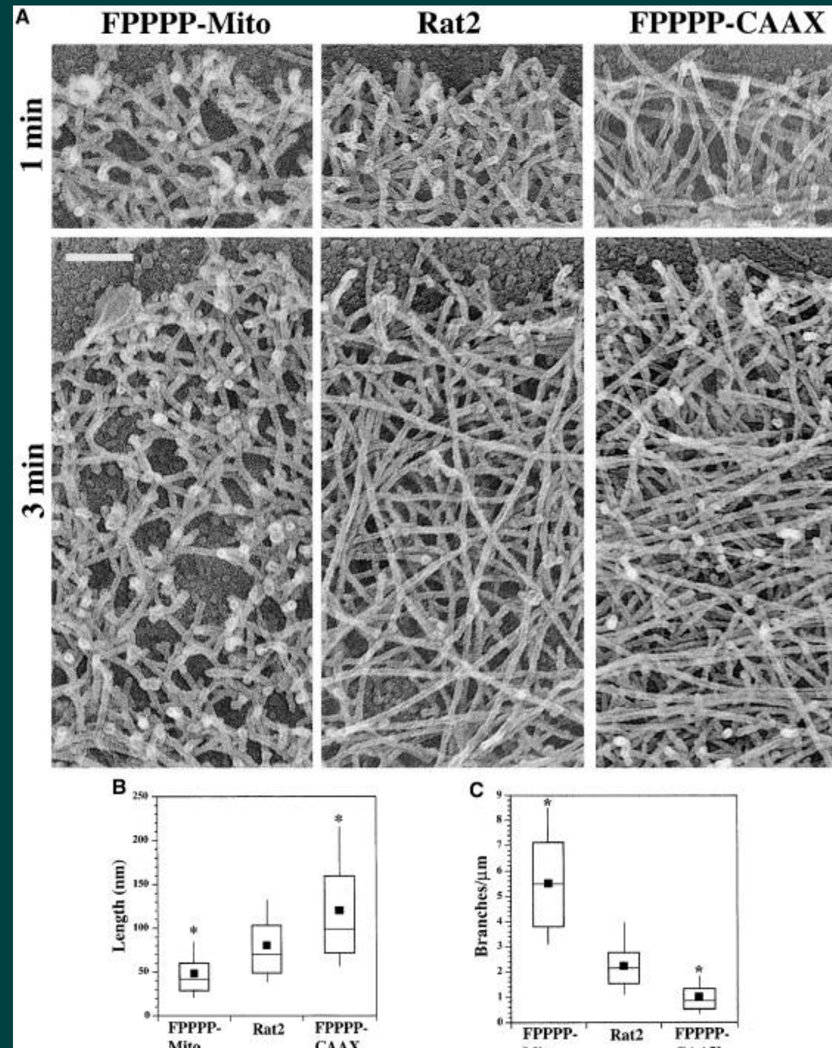
almost all of them!

Useful Proteins :

Profilin	2 µM
α-actinin	0.5 µM
VASP	0.1 µM

VASP increases actin filament length and protrusion speed but decreases protrusion persistence

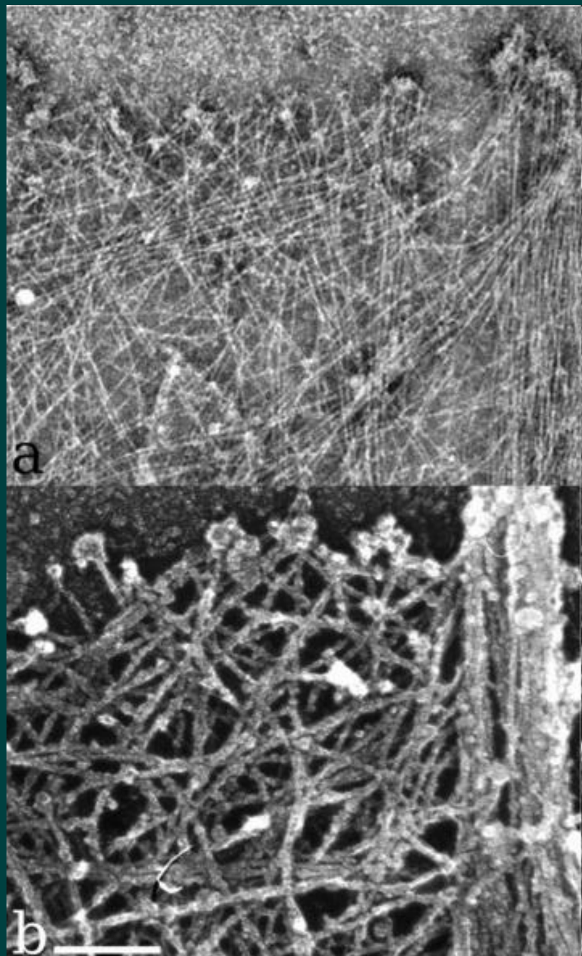




Bear et al., Cell, 2002

filament length is difficult to measure with electron microscopy

negative staining



freeze-drying/metal replication

electron tomography

Small et al., 2008

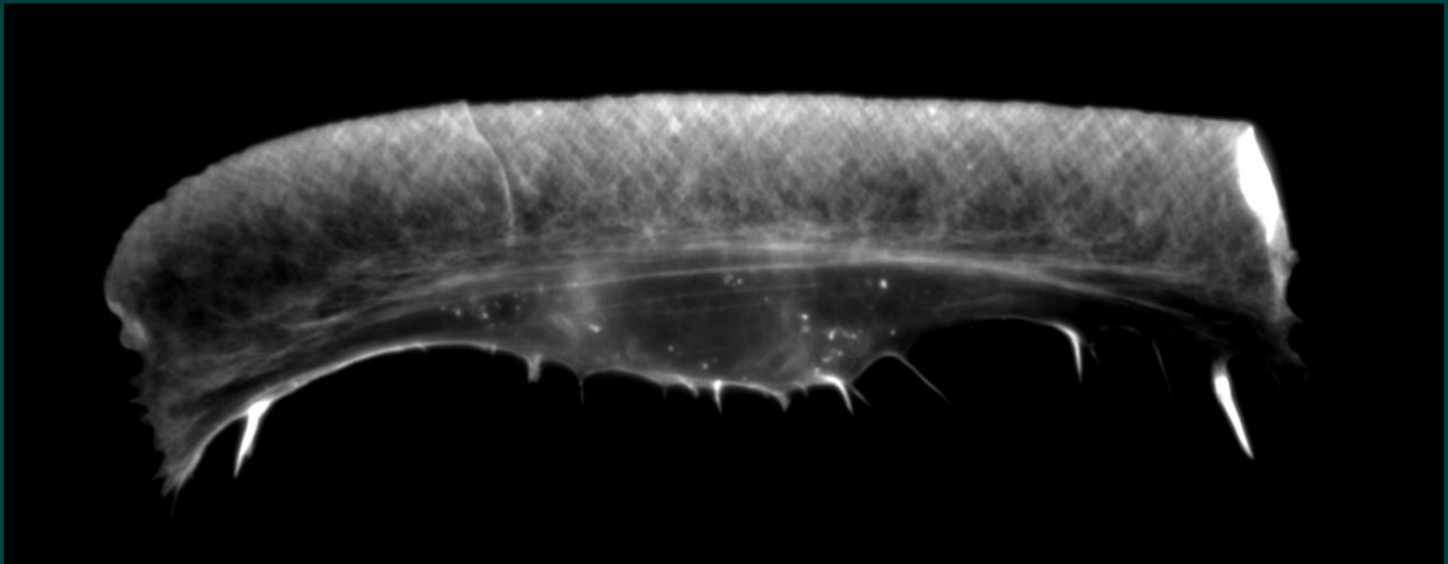
now:

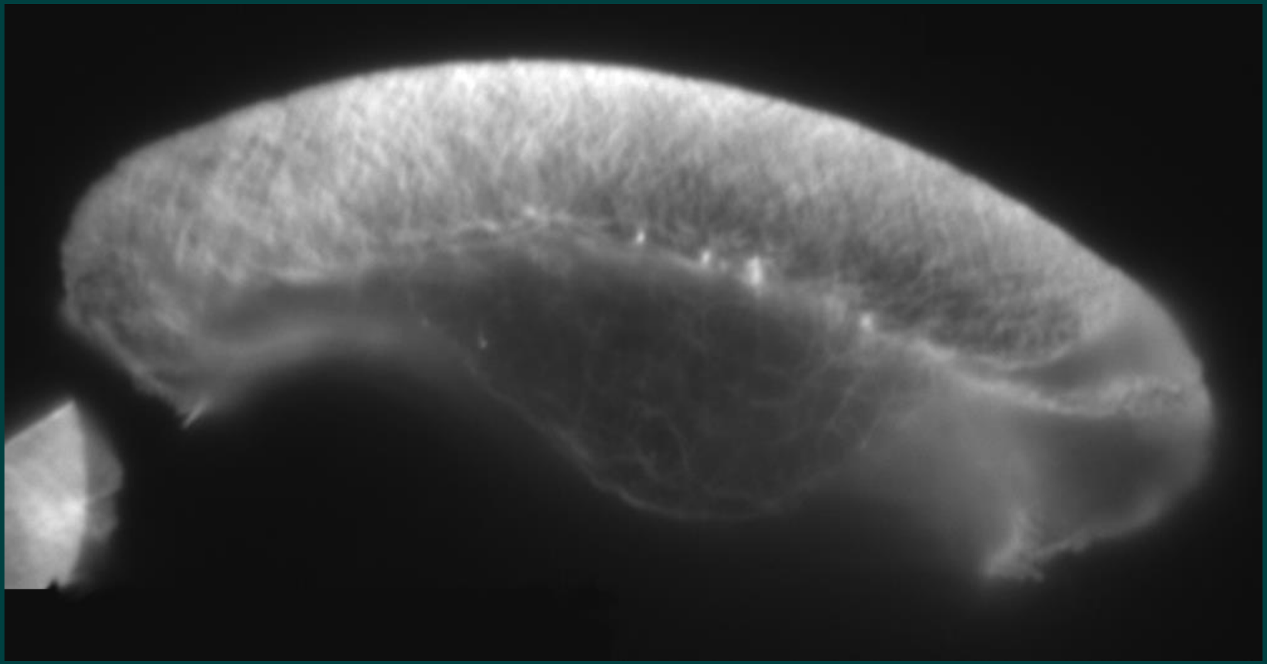
estimation of filament length with optical microscopy

how the cell makes long, unbranched filaments

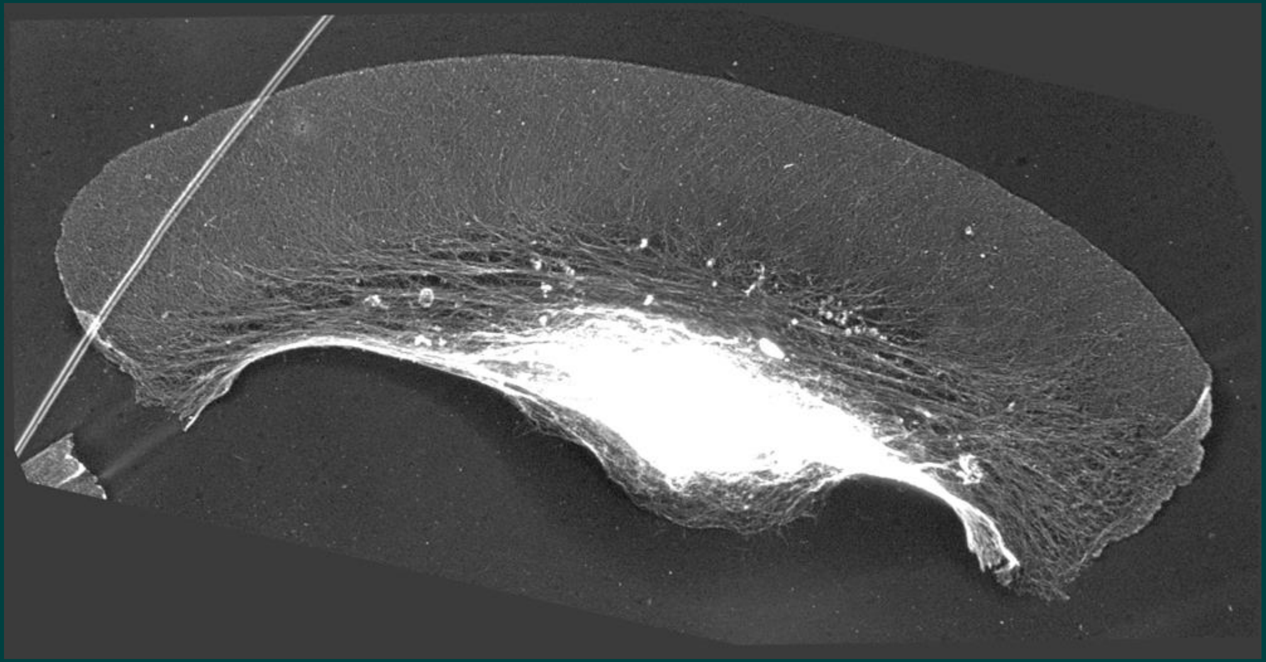
molecular motors

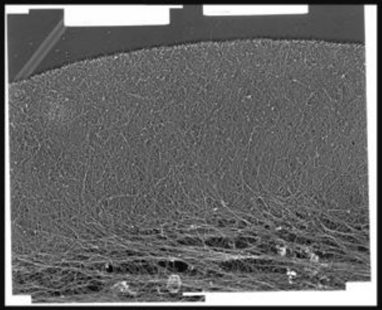
criss-cross pattern in the lamellipodia

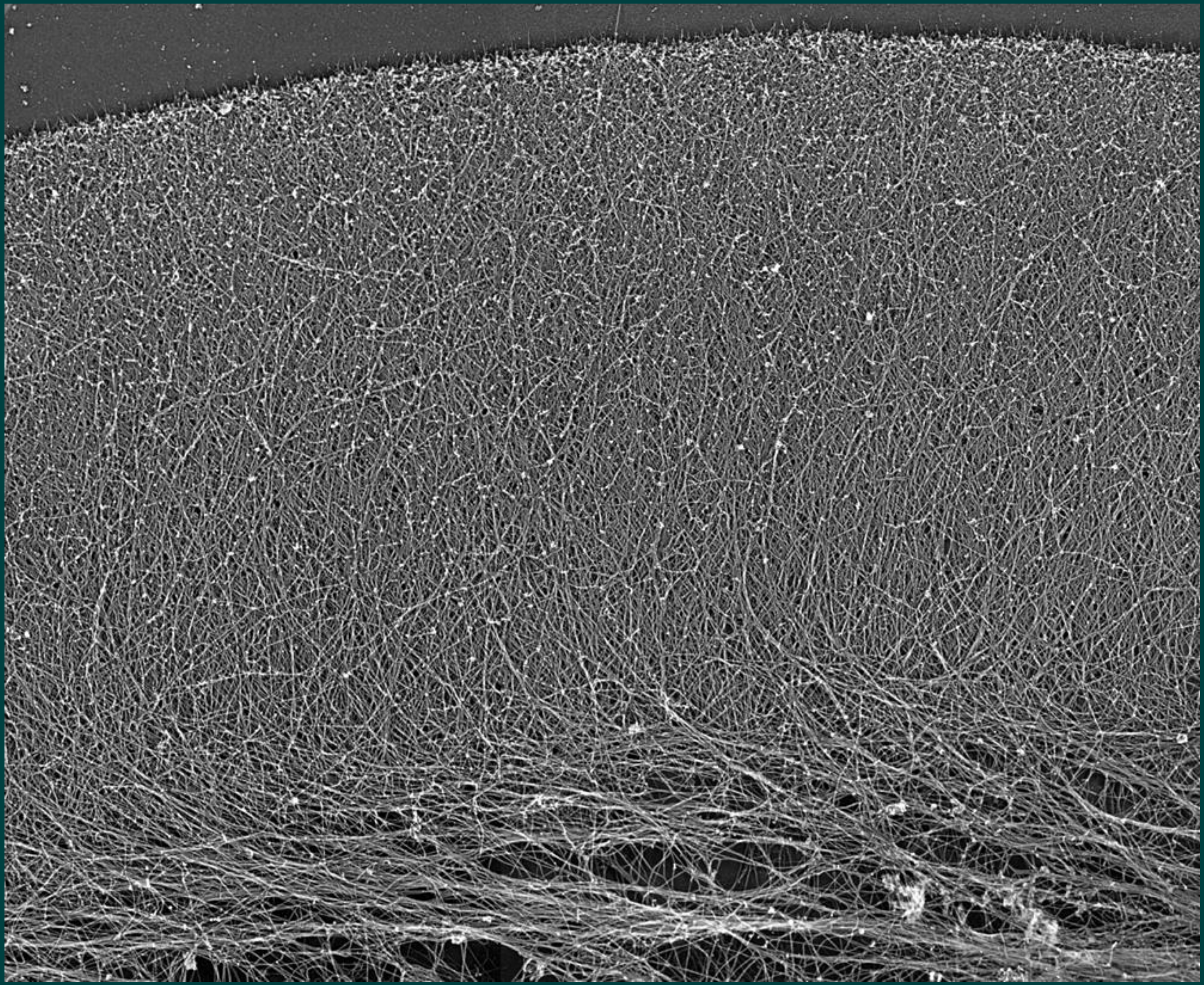


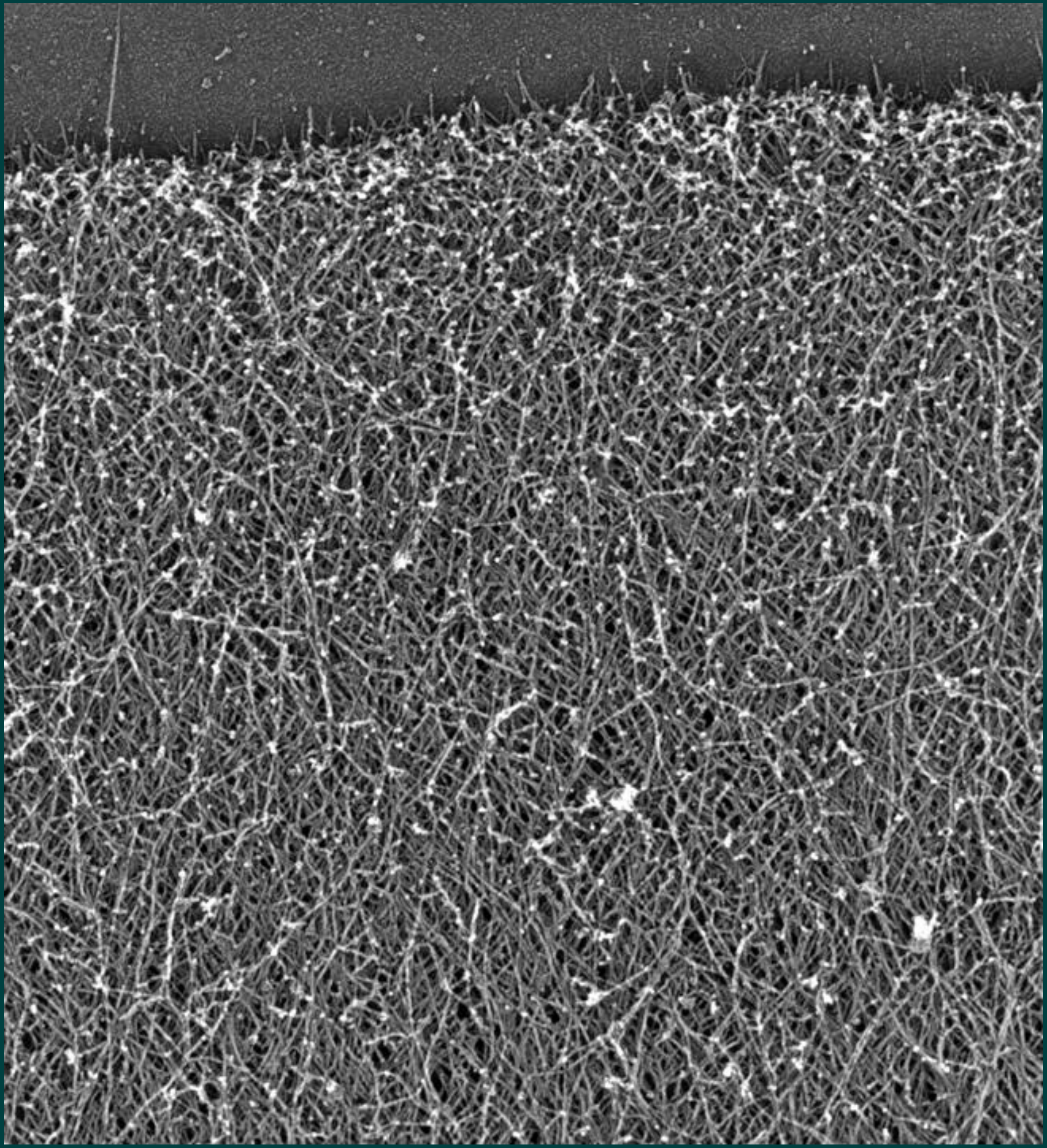


EM: platinum replica

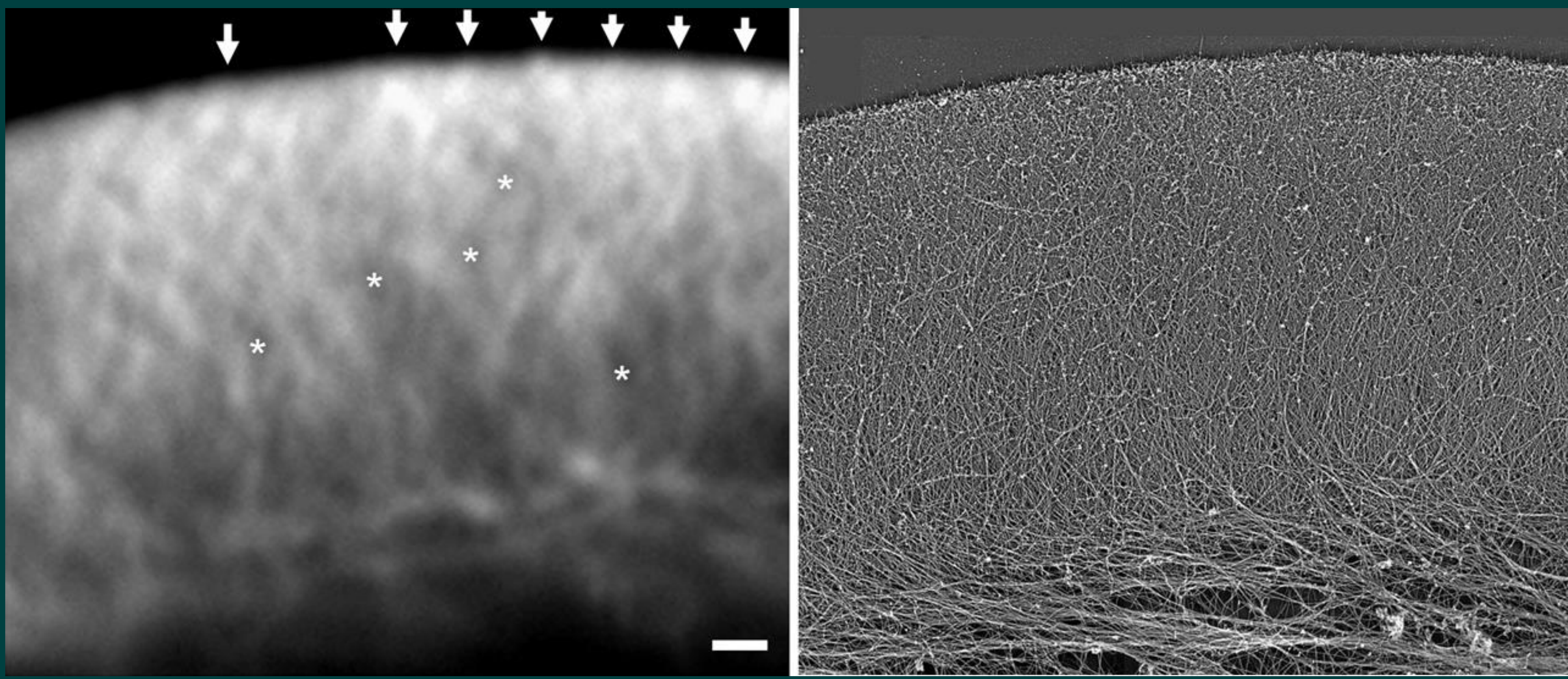


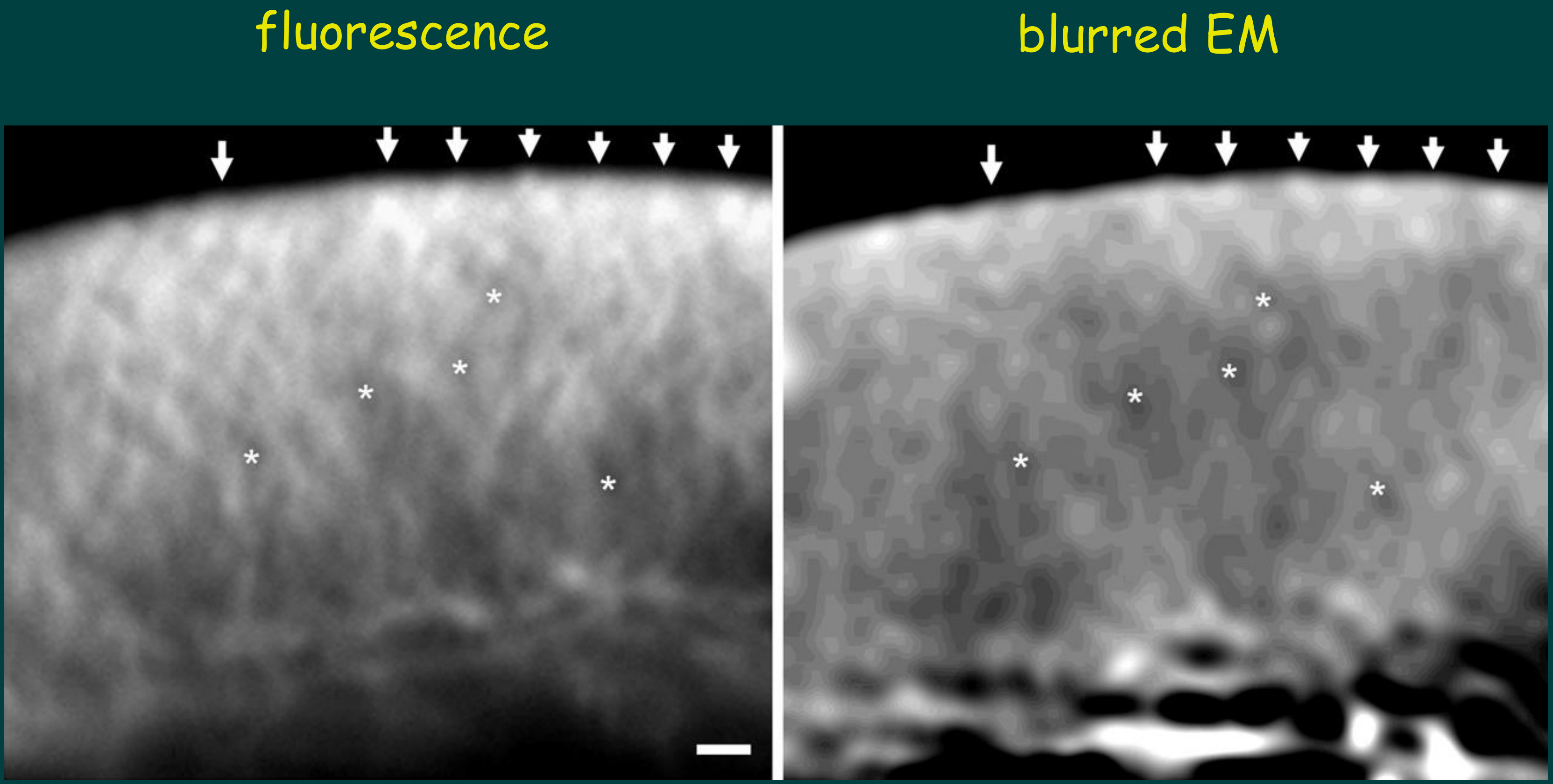






fluorescence | EM

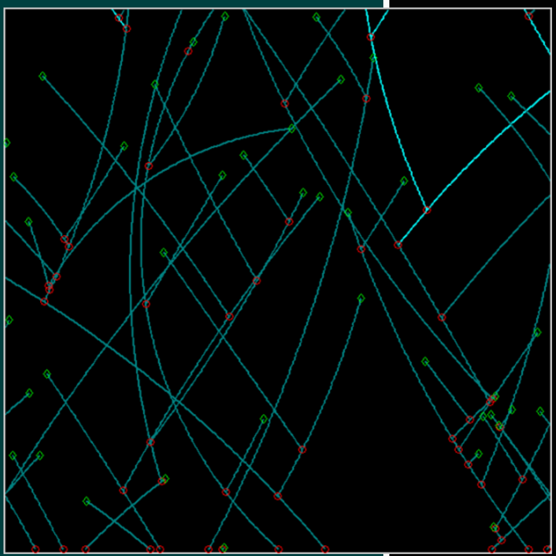




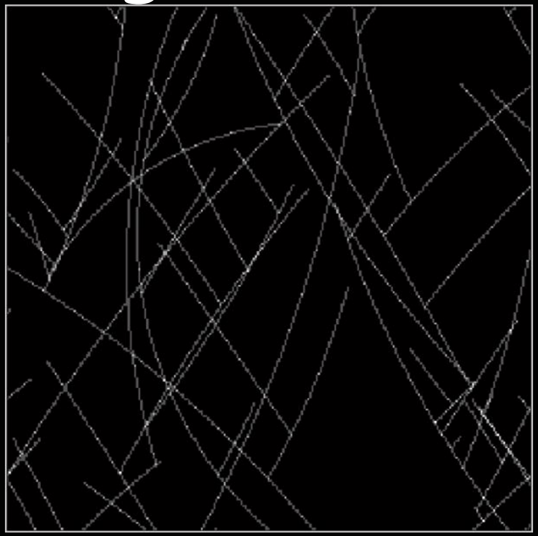
blurred EM corresponds well to fluorescence pattern:
pattern is due to filament density variation

Verkhovsky et al. Mol. Biol. Cell, 2003

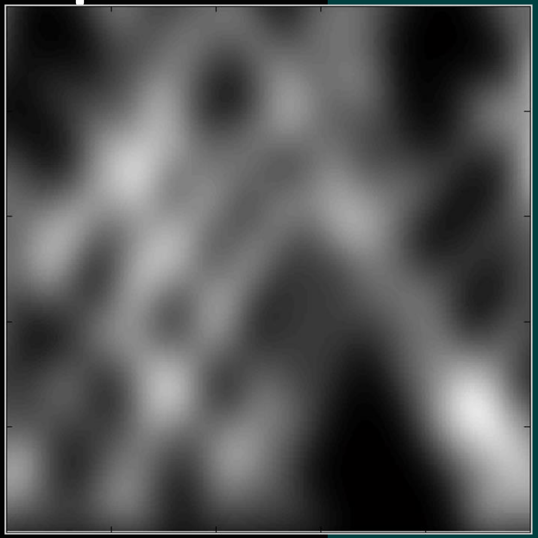
Simulating images of the lamellipodia

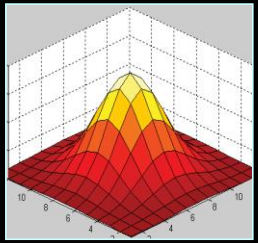


- Growing filament
- Capped filament
- Arp2/3
- Capping protein



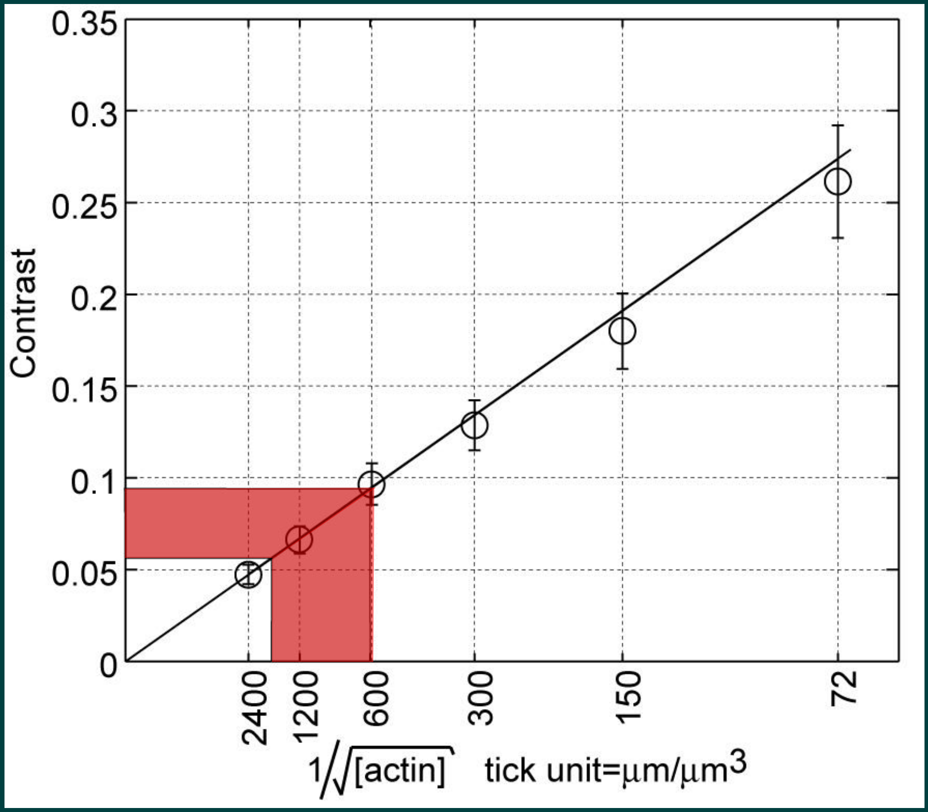
density map





diffraction spot of
our optical system

Image contrast: estimation of filament concentration



Contrast ∝ St.D./ Mean

$$=\beta/\sqrt{[actin]}$$

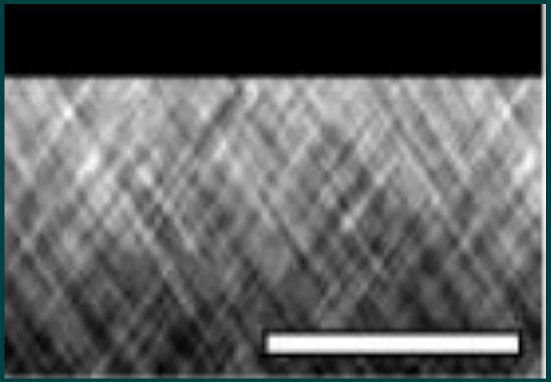
β =0.18

➔Concentration = 1160 ± 550 µm/µm³ = 900 ± 400 µM

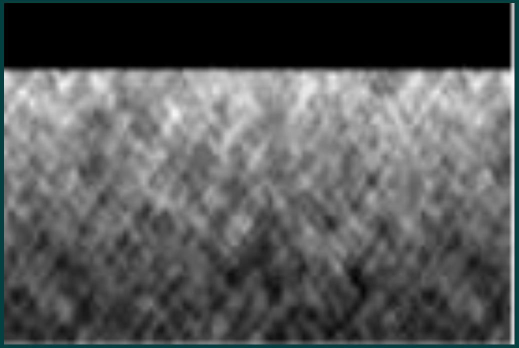
***(1580 ± 613 µm/µm³, Abraham et al. Biophys. J.* 1999)**

Image texture : visual comparison

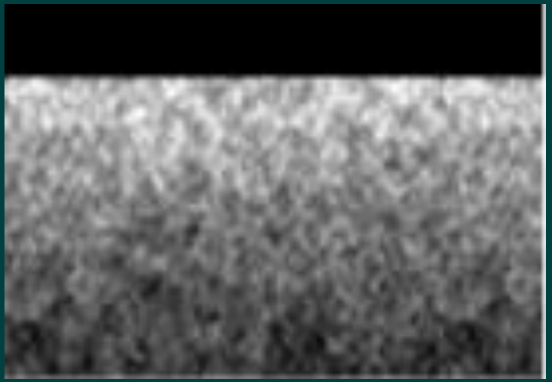
L=4μm R=500μm δθ=1°



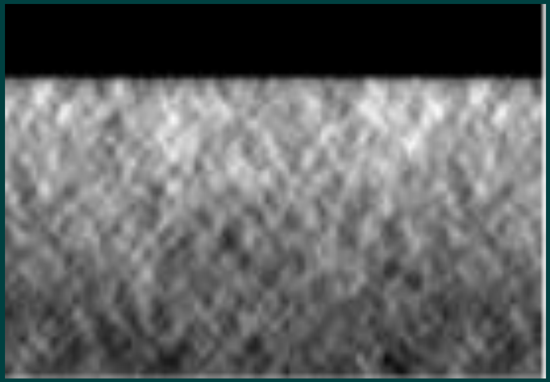
L=1μm R=500μm δθ=1°



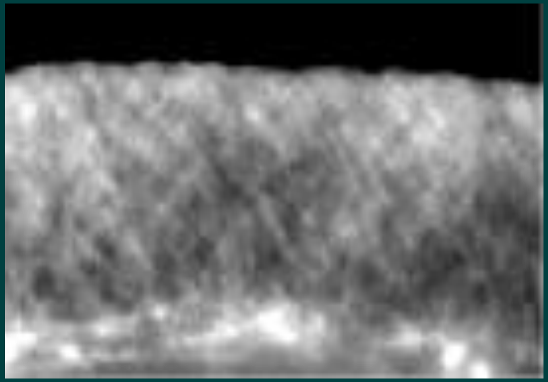
L=0.3μm R=500μm δθ=1°



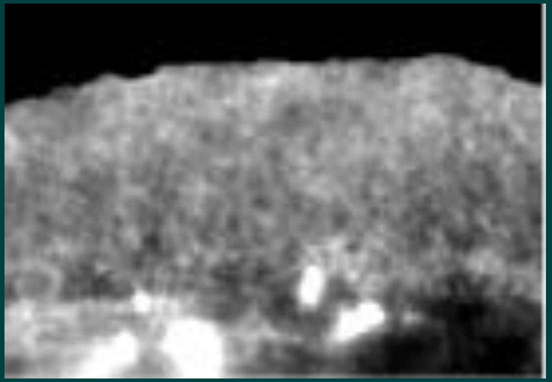
L=4μm R=10μm δθ=20°



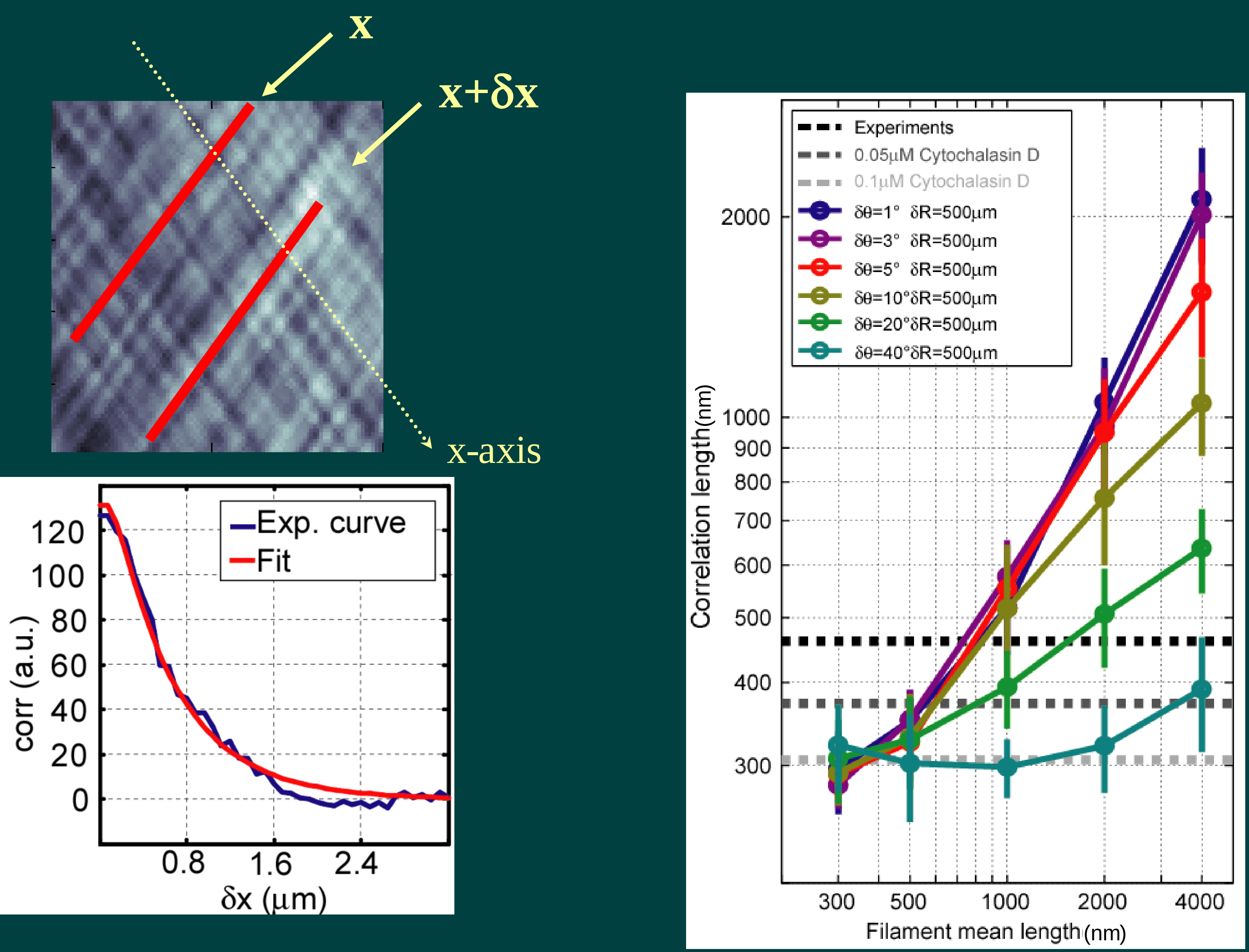
Experimental



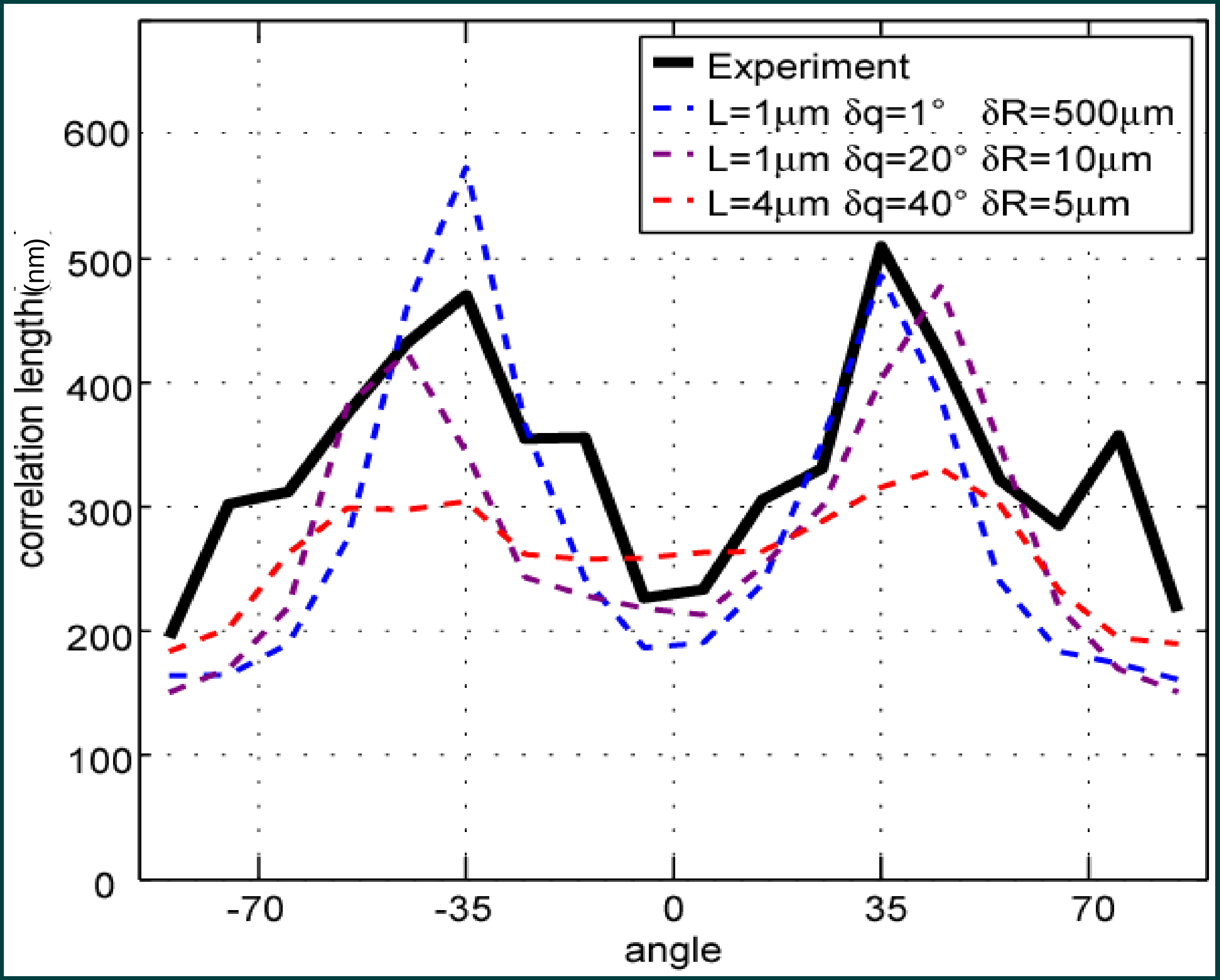
Exp. +0.1μM cytochalasin D



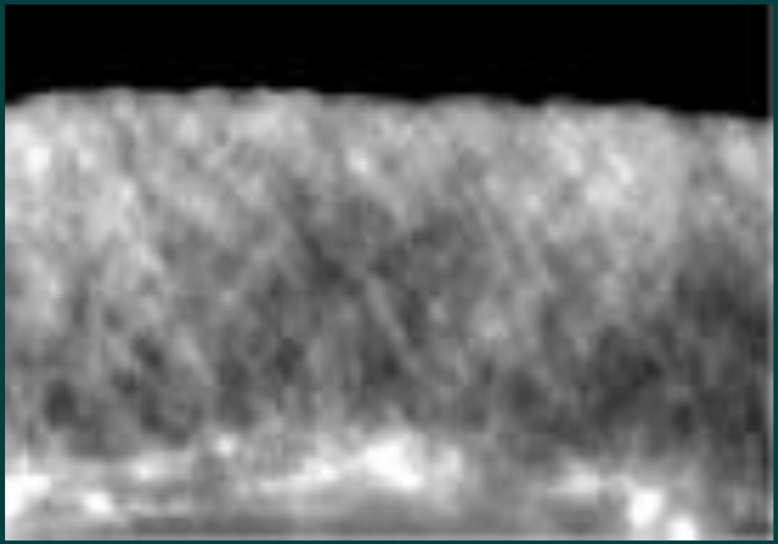
Correlation length



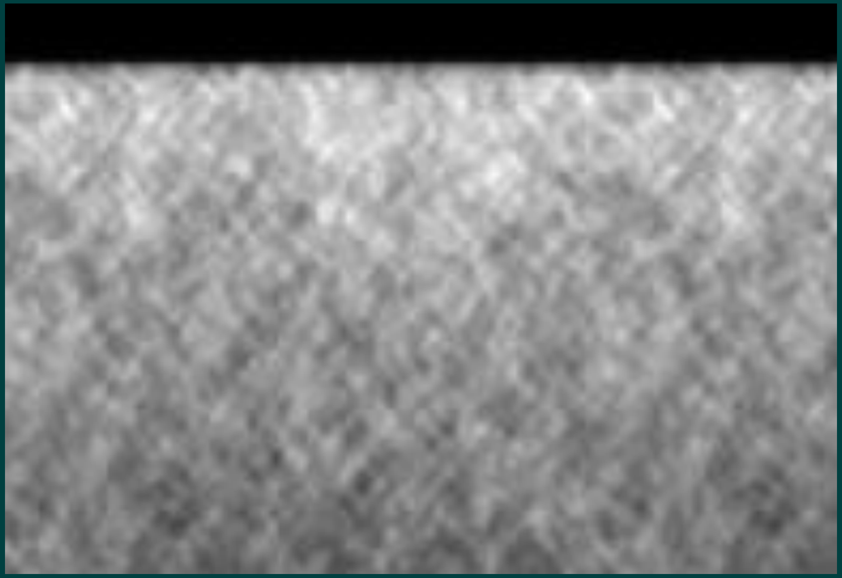
Orientational distribution of correlation length



Experimental



Simulations



Filament Mean Length=1.3μm

Curvature Radius=10 μm

Branching Fluctuation=20°

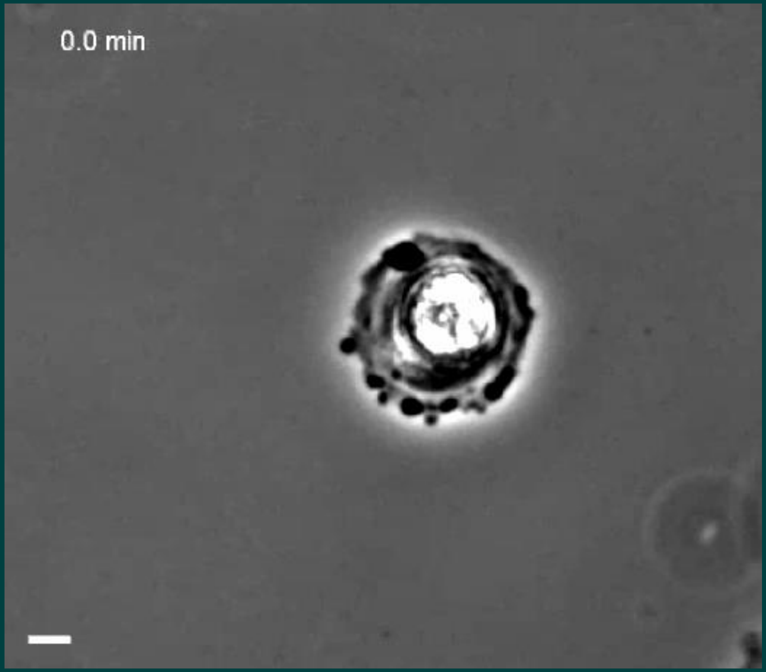
Capping Rate≅0.23 s^{-1}

Depolymerizing Rate ≅ 0.015s^{-1}

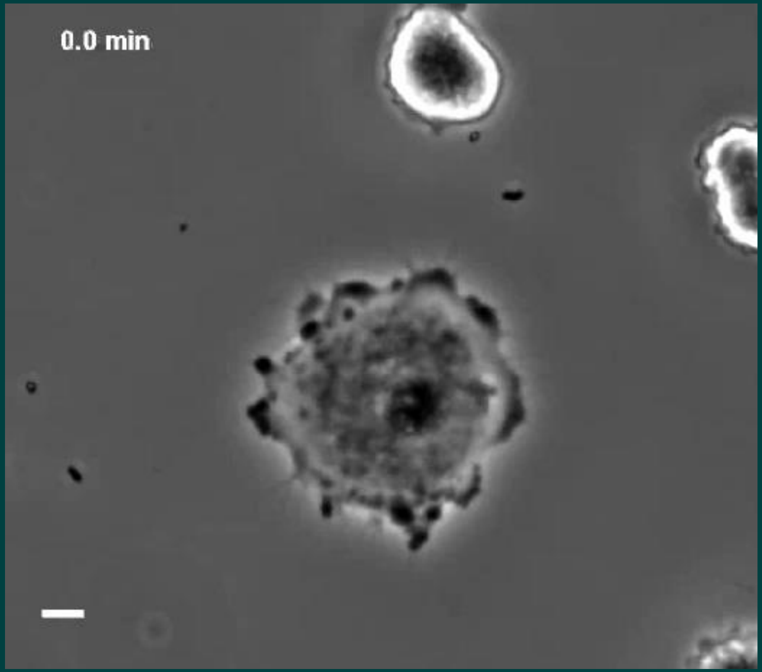
Schaub et al., J. Cell Sci., 2007

Arp2,3 is essential for lamellipodia, but not essential for cell spreading

ARPC3+/+

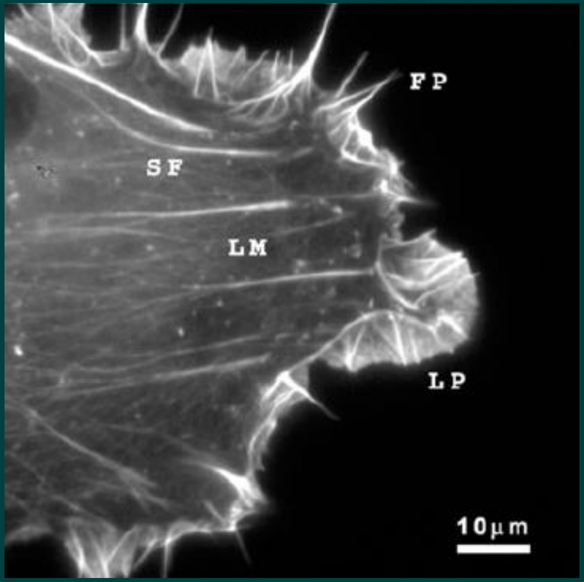


ARPC3-/-



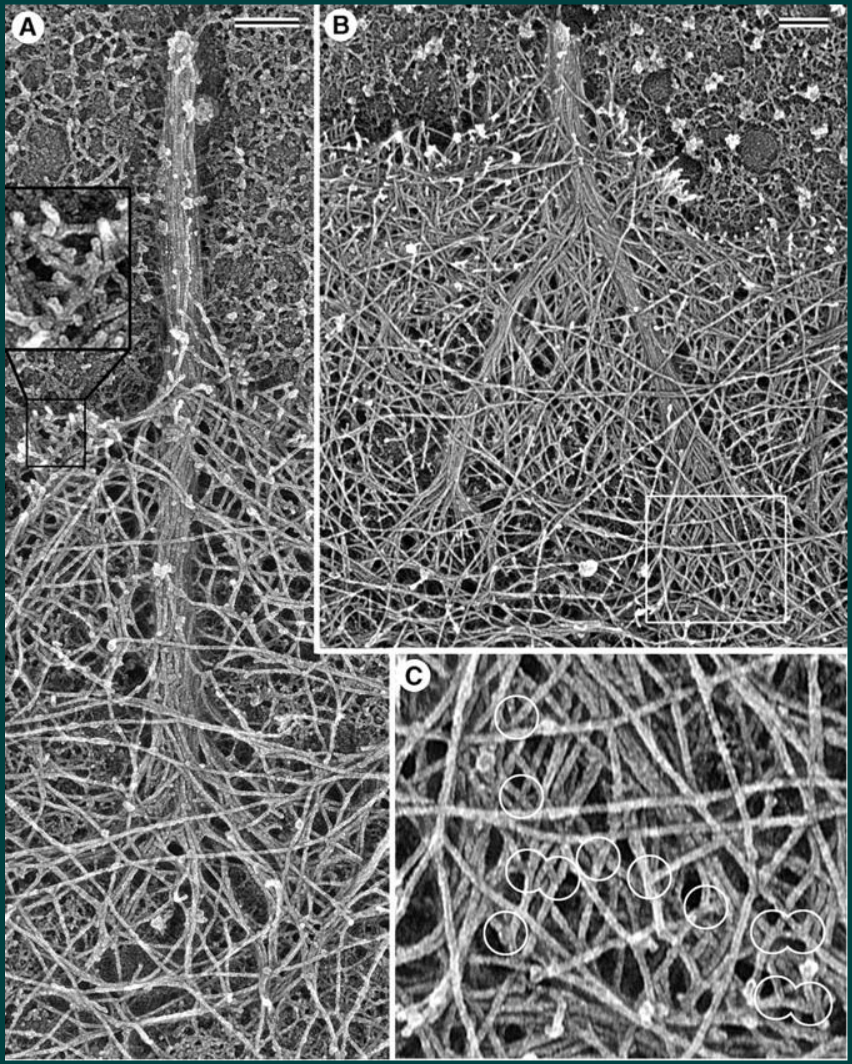
Suraneni et al., JCB, 2012

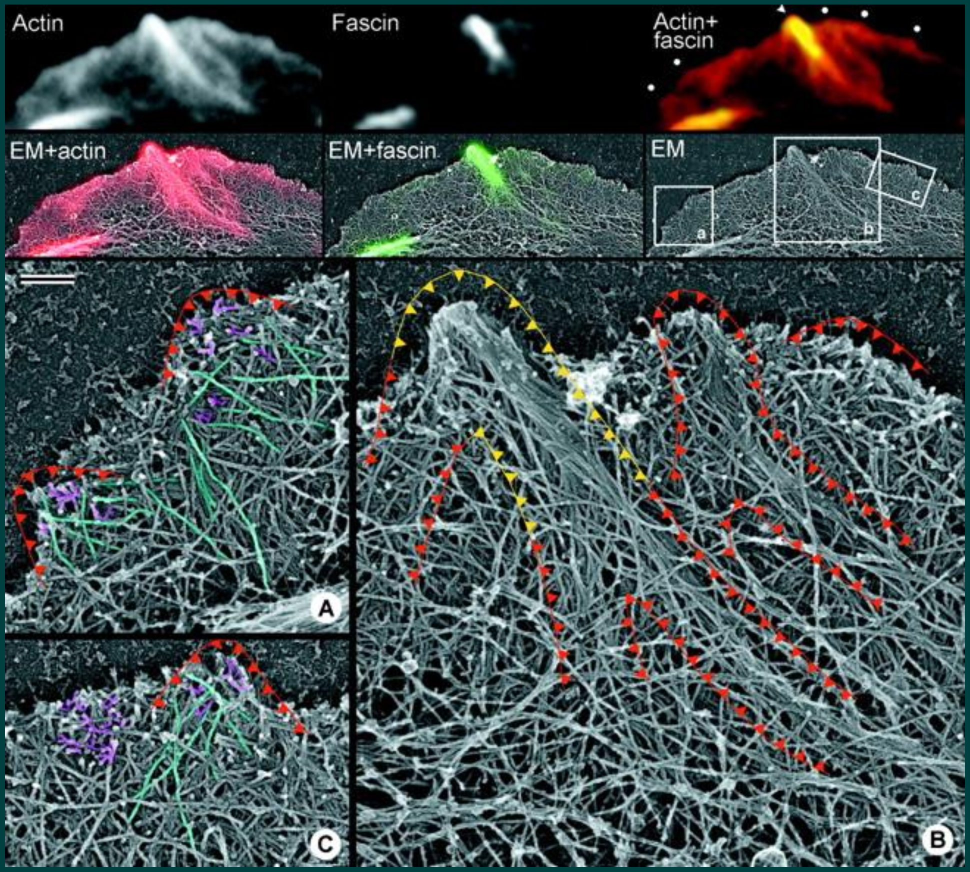
There are other actin structures in the cell, besides lamellipodium: filopodia, stress fibers in the lamellum



How do they form?

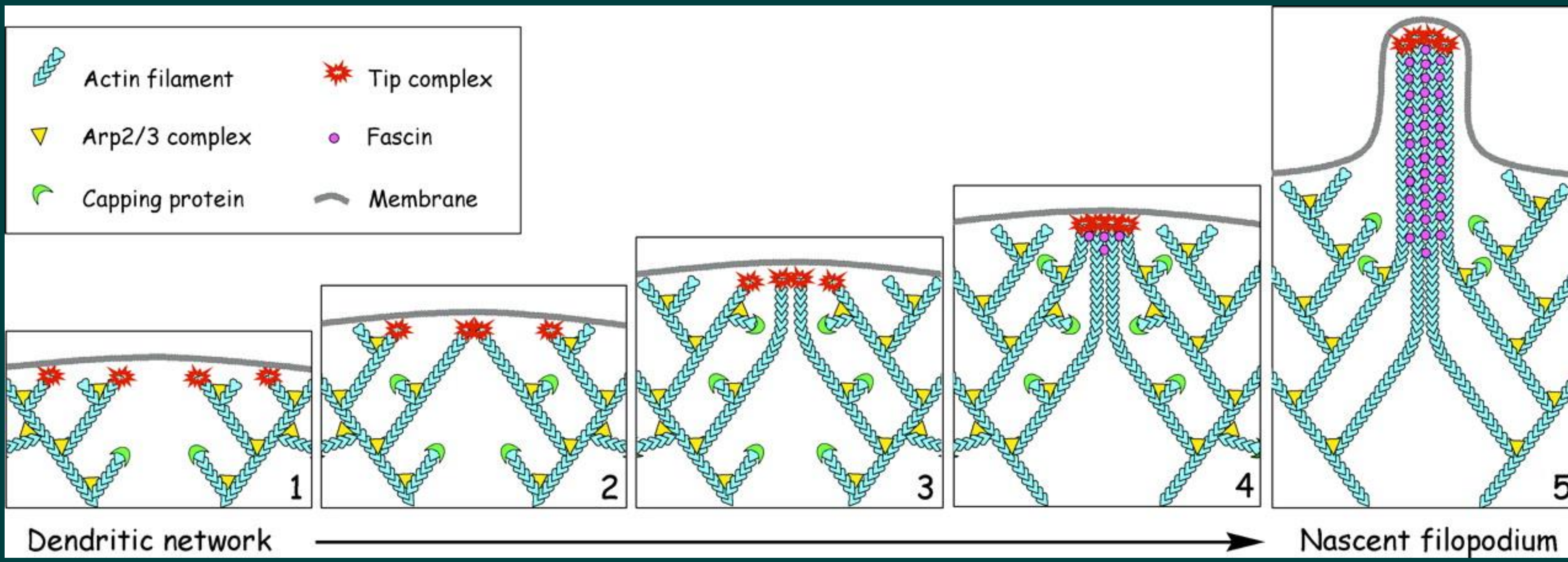
Mechanism of filopodia extension from lamellipodial network





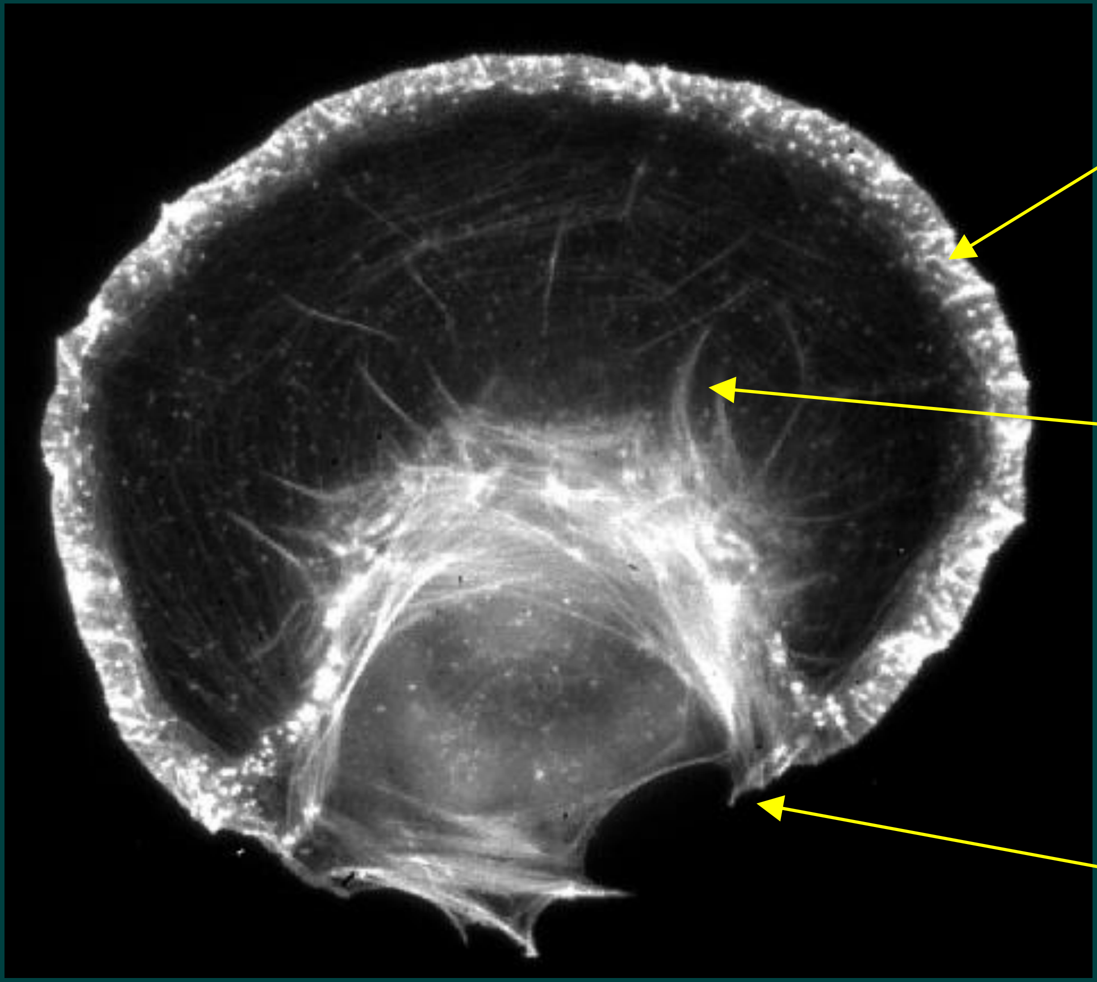
Svitkina TM et al. J Cell Biol. 2003 Feb 3;160(3):409-21.

Mechanism of filopodia extension



Svitkina TM et al. J Cell Biol. 2003 Feb 3;160(3):409-21.

Stress fibers seems to assemble independently of the lamellipodia

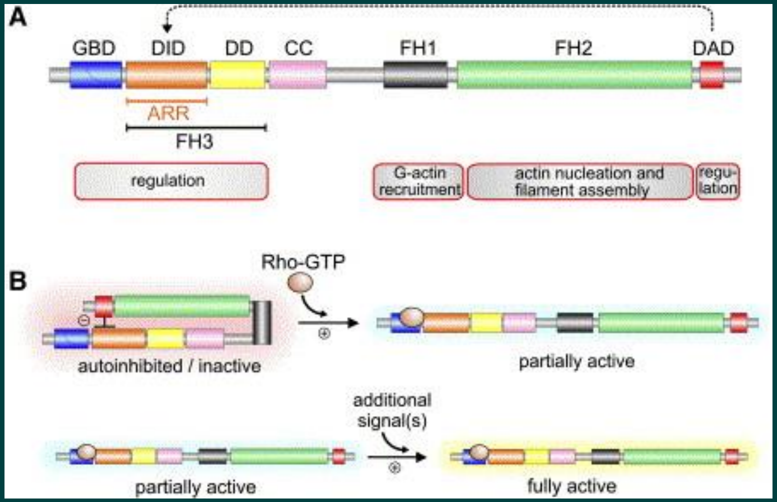


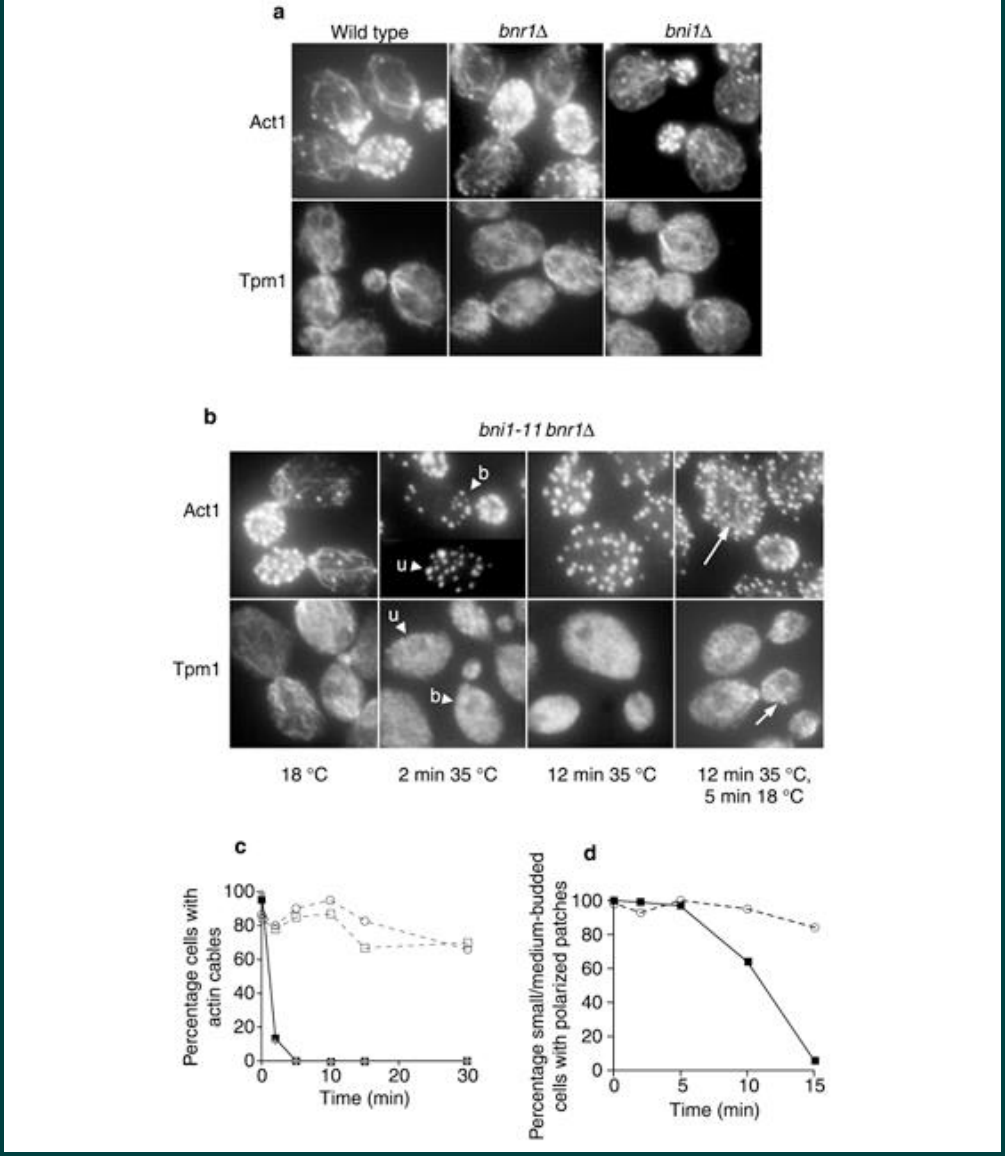
lamellipodia

stress fibers

filopodia

Actin patches and cables in yeast: cables require formins

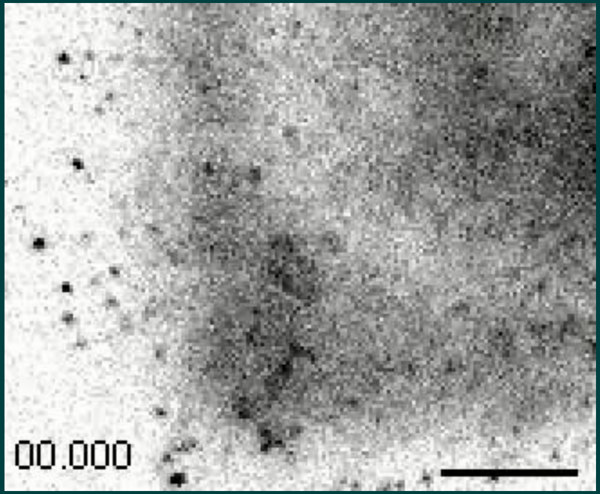


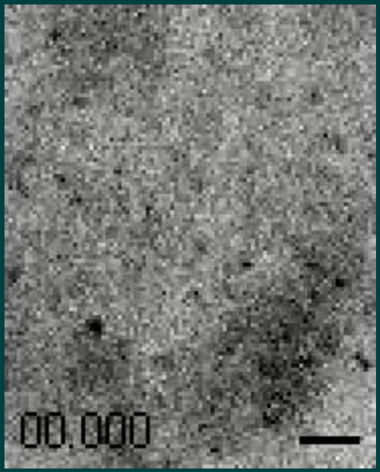


M. Evangelista, D. Pruyne, D.C. Amberg, C. Boone and A. Bretscher,

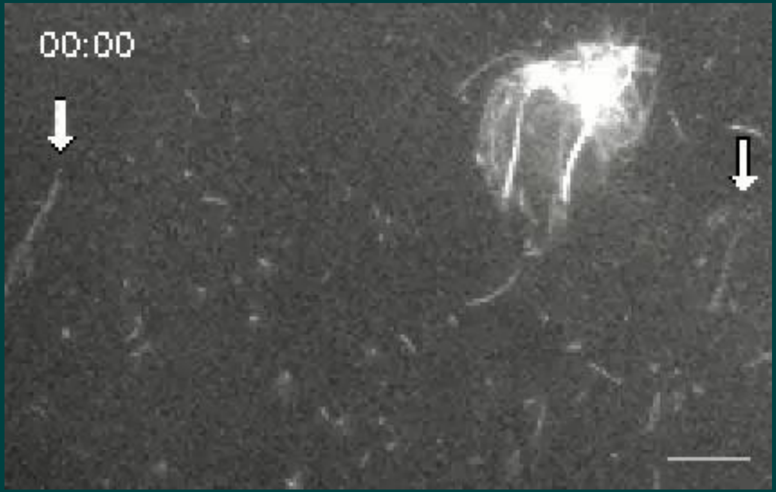
Formins direct Arp2/3-independent actin filament assembly to polarize cell growth in yeast. *Nat Cell Biol* **4** (2002), pp. 32-41

Processive movement of formin mDia in animal cells by single molecule FSM



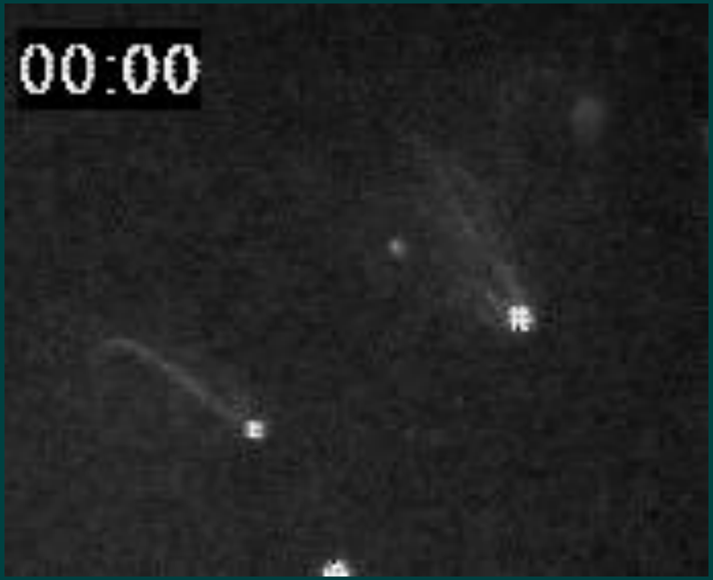


Cytochalasin D



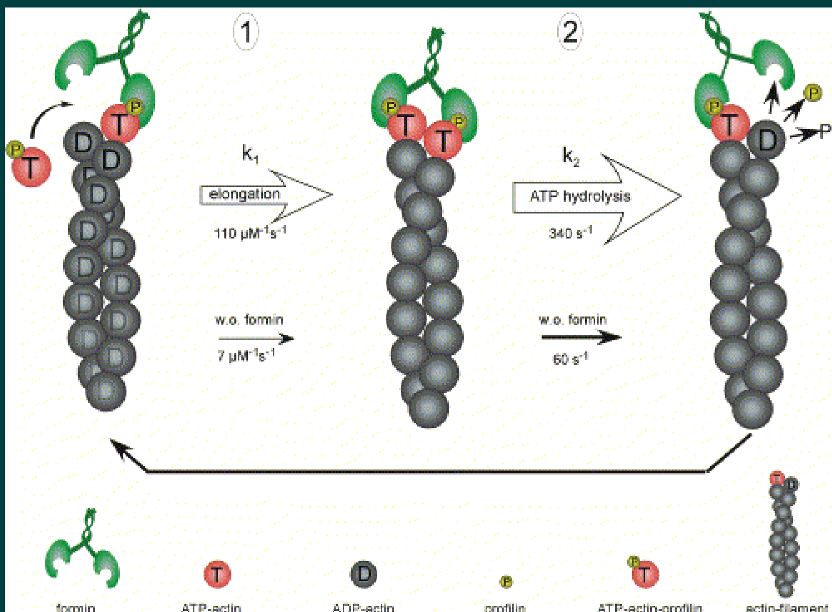
C. Higashida, T. Miyoshi, A. Fujita, F. Oceguera-Yanez, J. Monypenny, Y. Andou, S. Narumiya and N. Watanabe, Actin polymerization-driven molecular movement of mDia1 in living cells. Science 303 (2004), pp. 2007–2010.

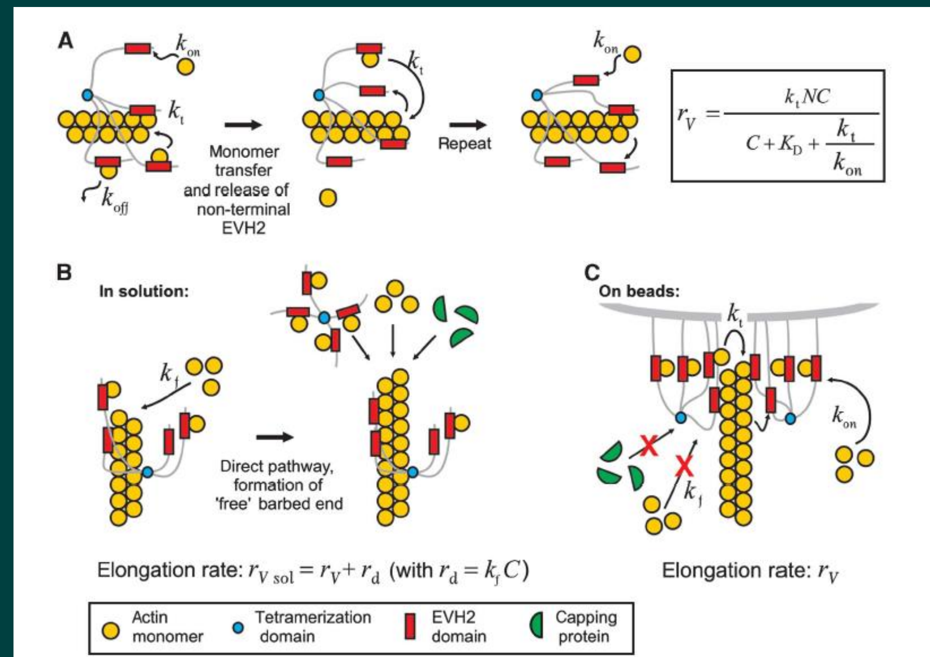
Reconstruction of formin-stimulated motility in vitro



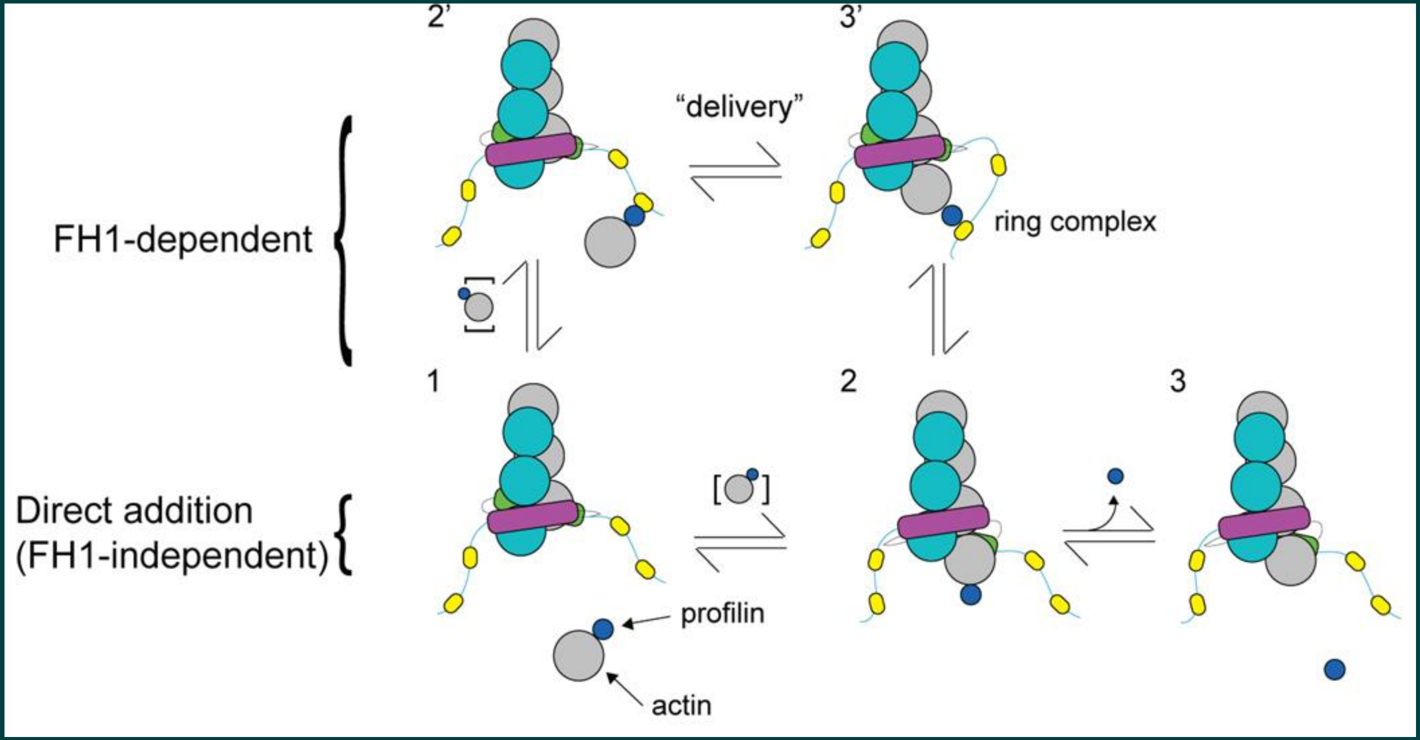
Romero et al., Cell, 2004

Formins and ENA-VASP family proteins work like end-tracking motors



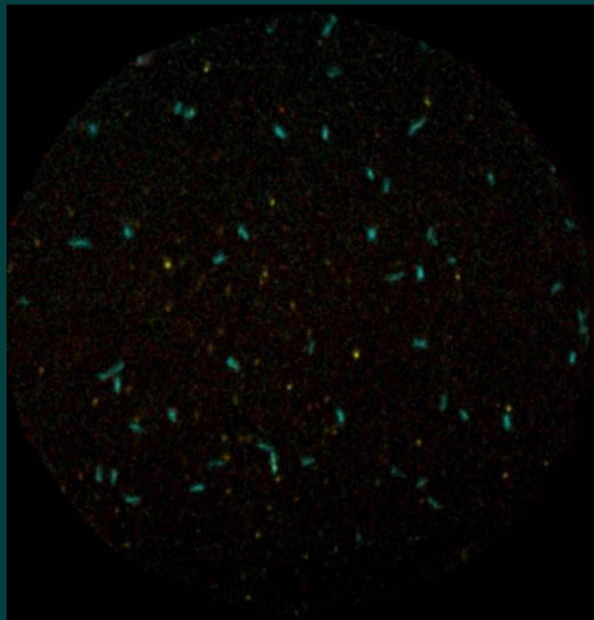


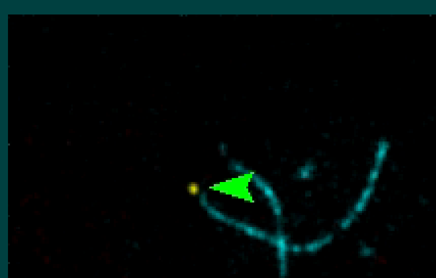
acceleration of actin elongation by delivery of profilactin complex by formin FH1 domains

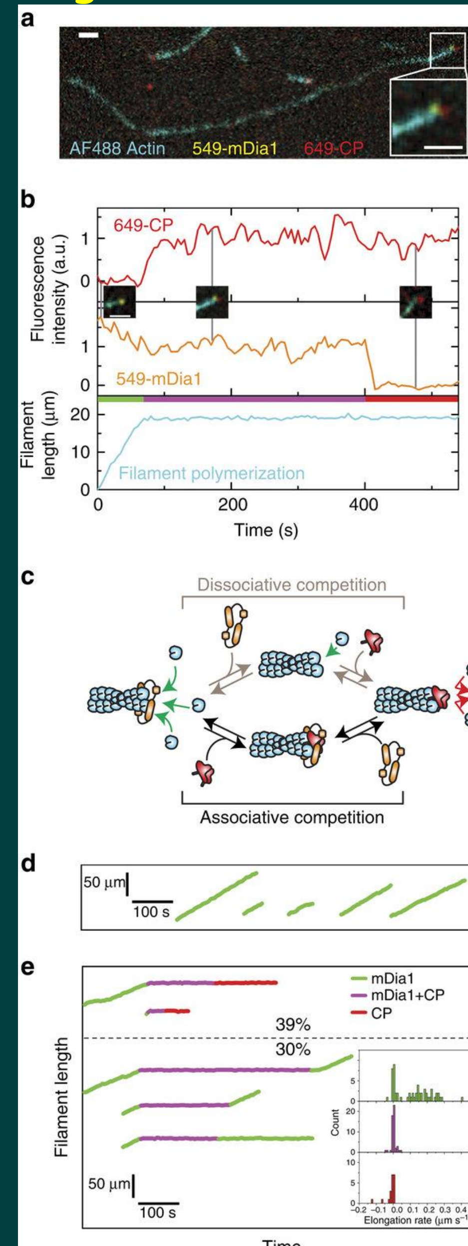


Paul and Pollard, 2009

Competition between formin and capping protein by single molecule fluorescence

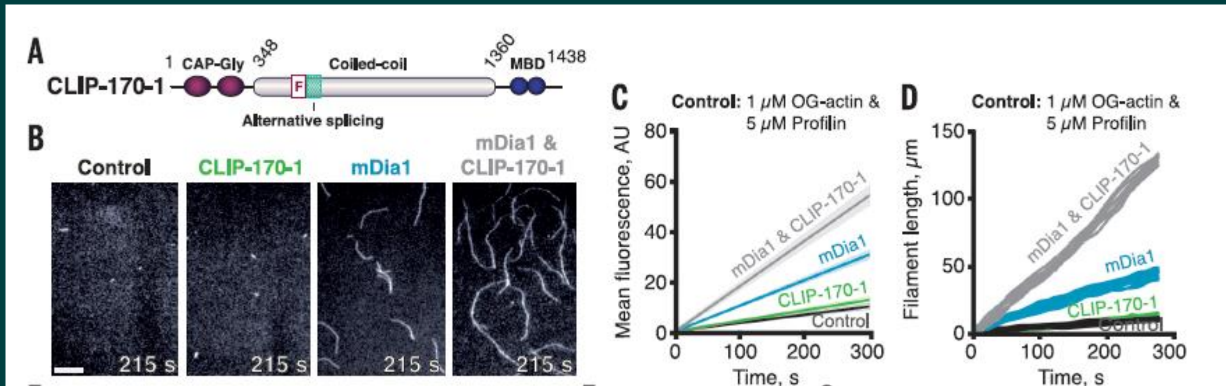


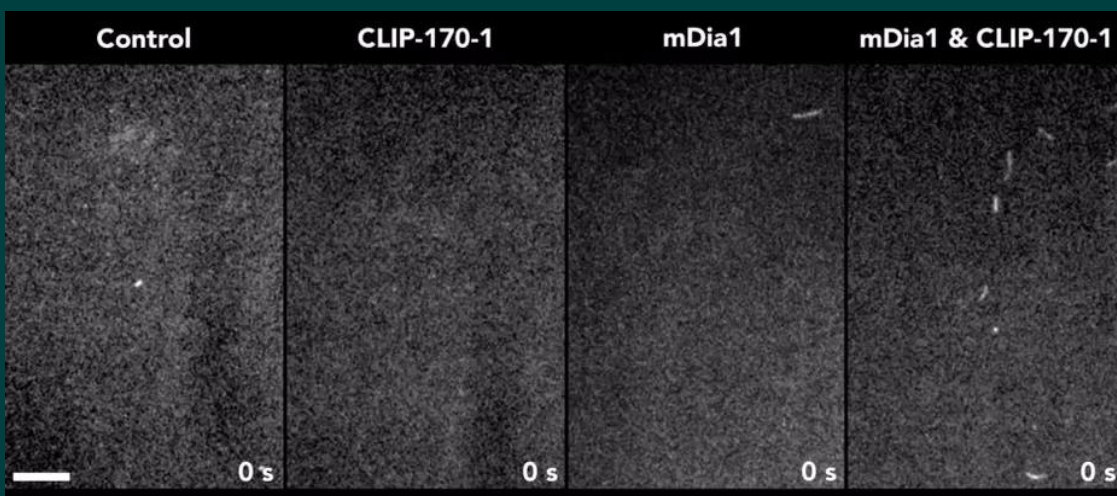


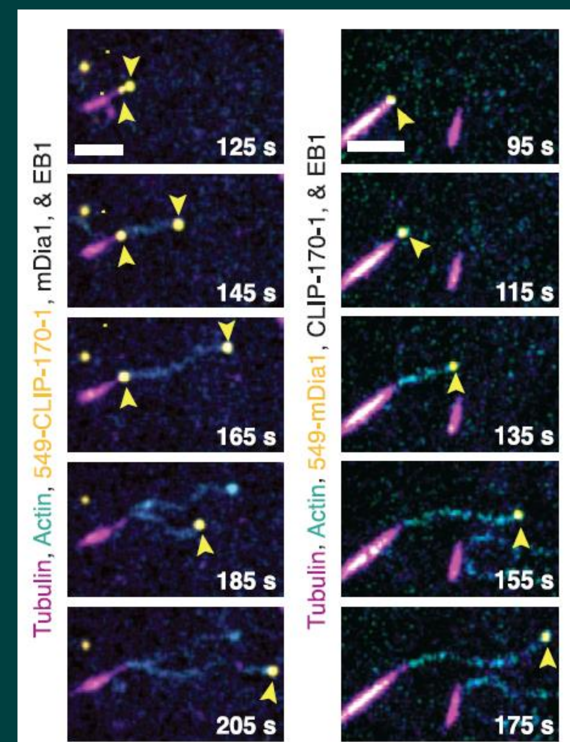


Bombardier et al., 2015

formin interacts with microtubule end-binding protein CLIP-170 to grow actin filaments from microtubule ends

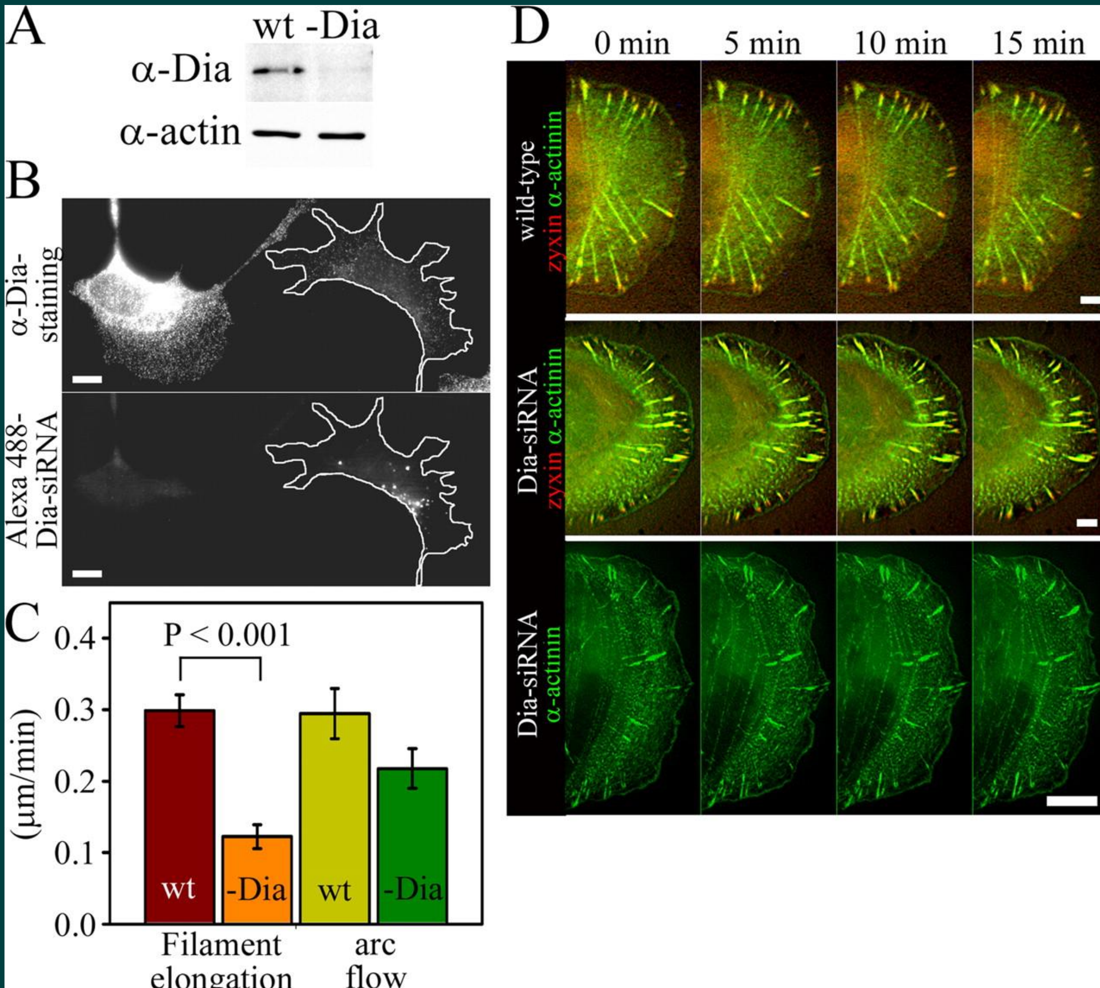


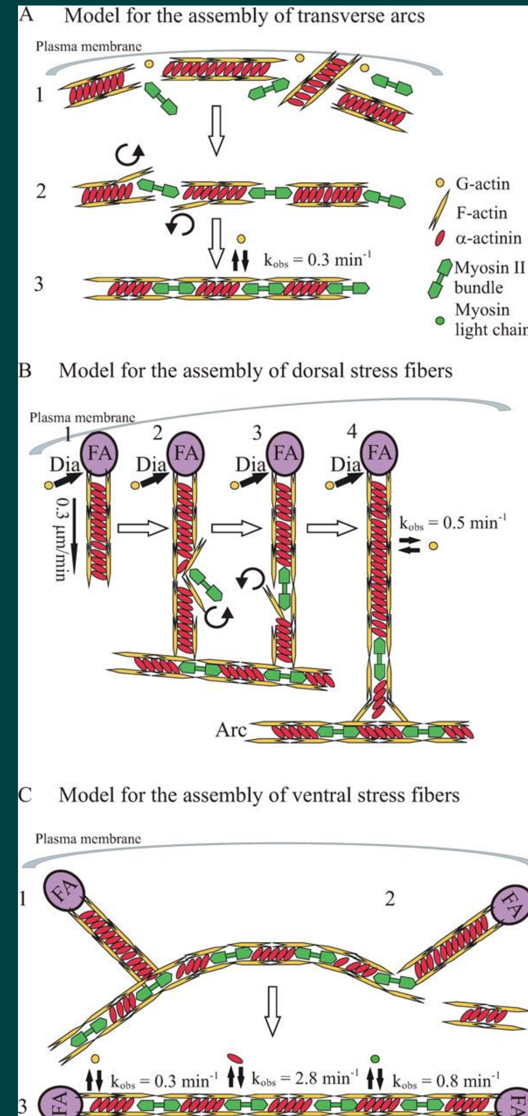




Henti-Ridilla et al., 2016

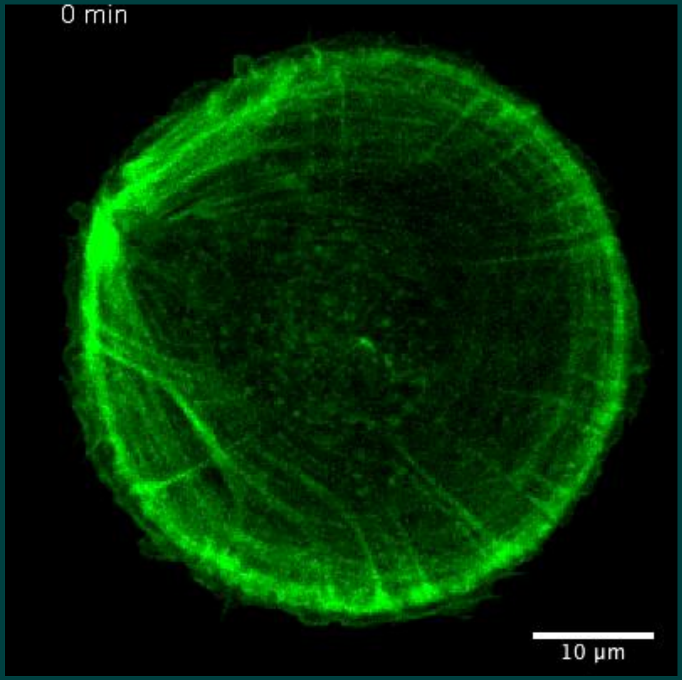
Formins (mDia) contribute to the assembly of stress fibers

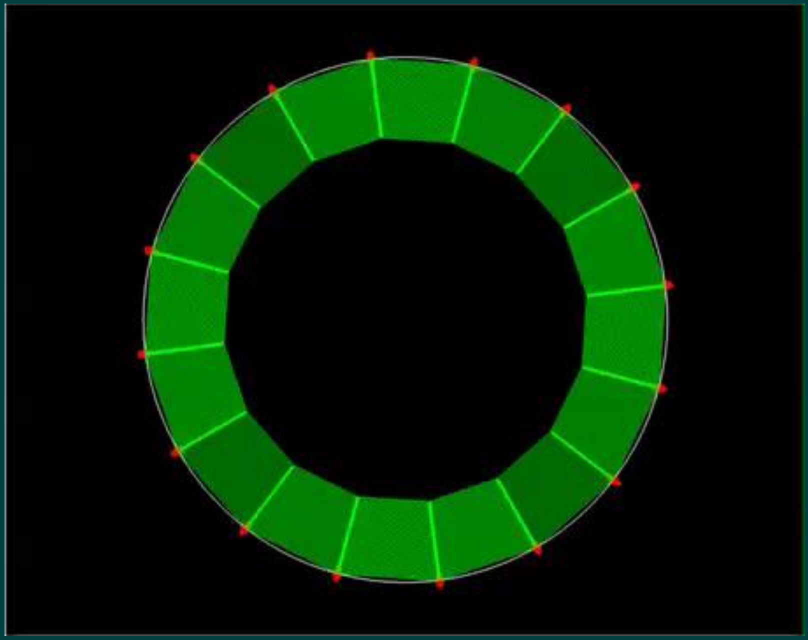




Hotulainen and Lappalainen, JCB, 2006

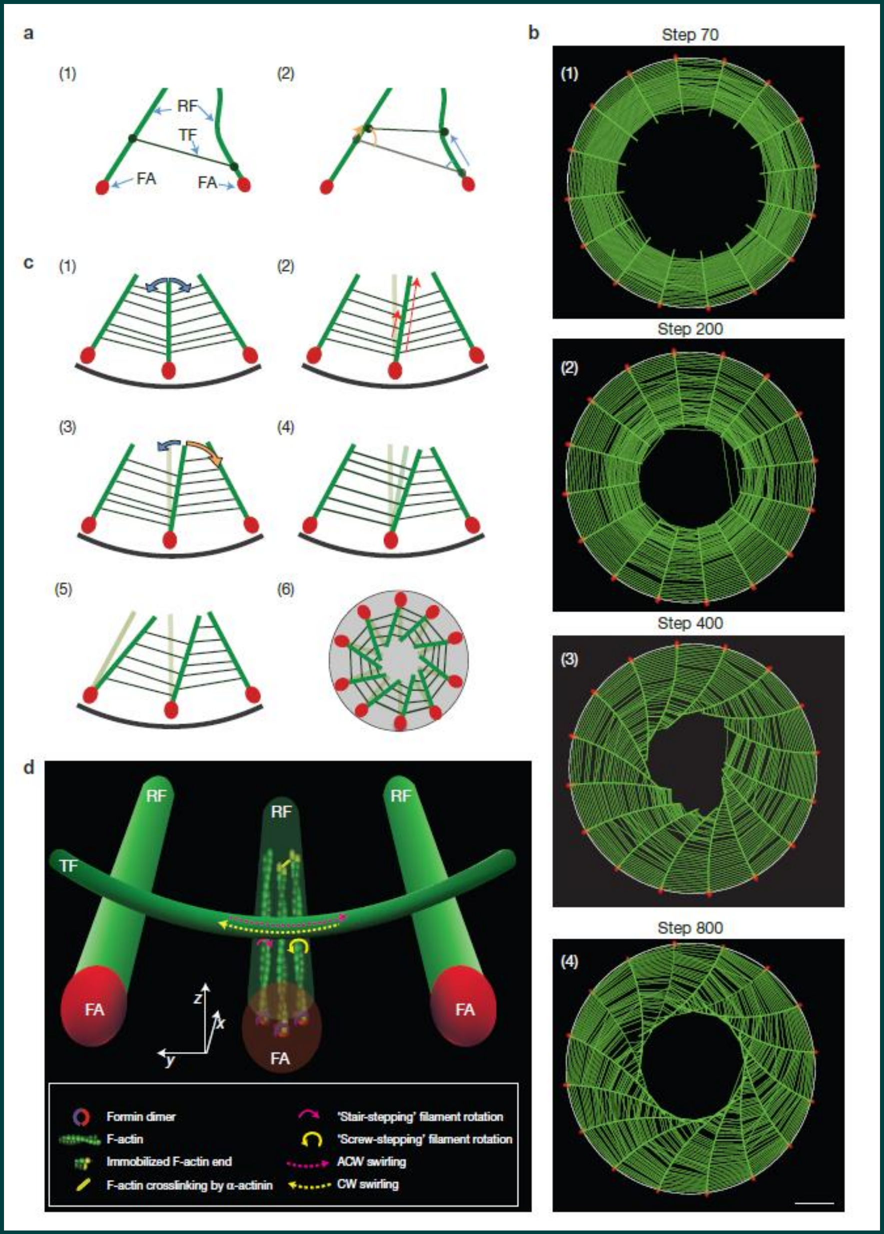
cellular chirality emerges from actin dynamics:
possible role of filament rotation while elongating from formins





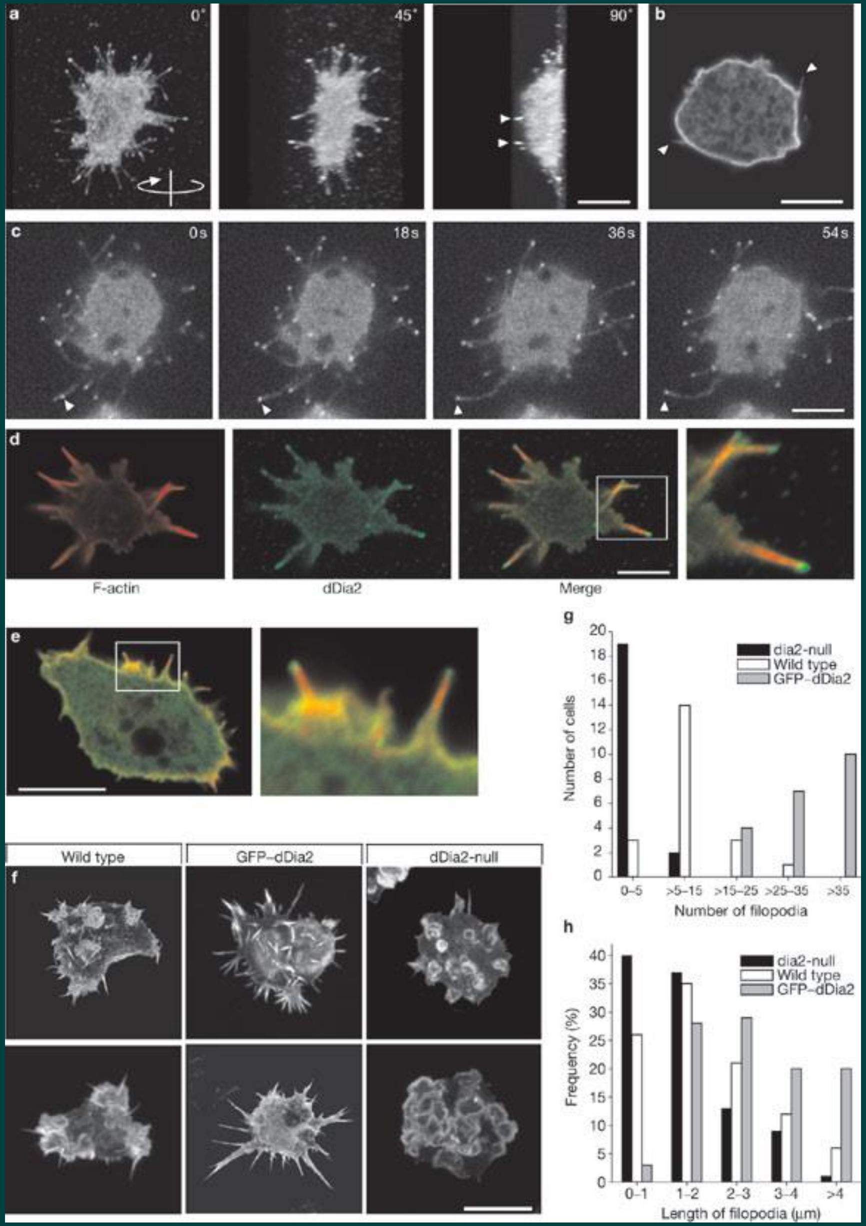
Tee et al., NCB, 2015

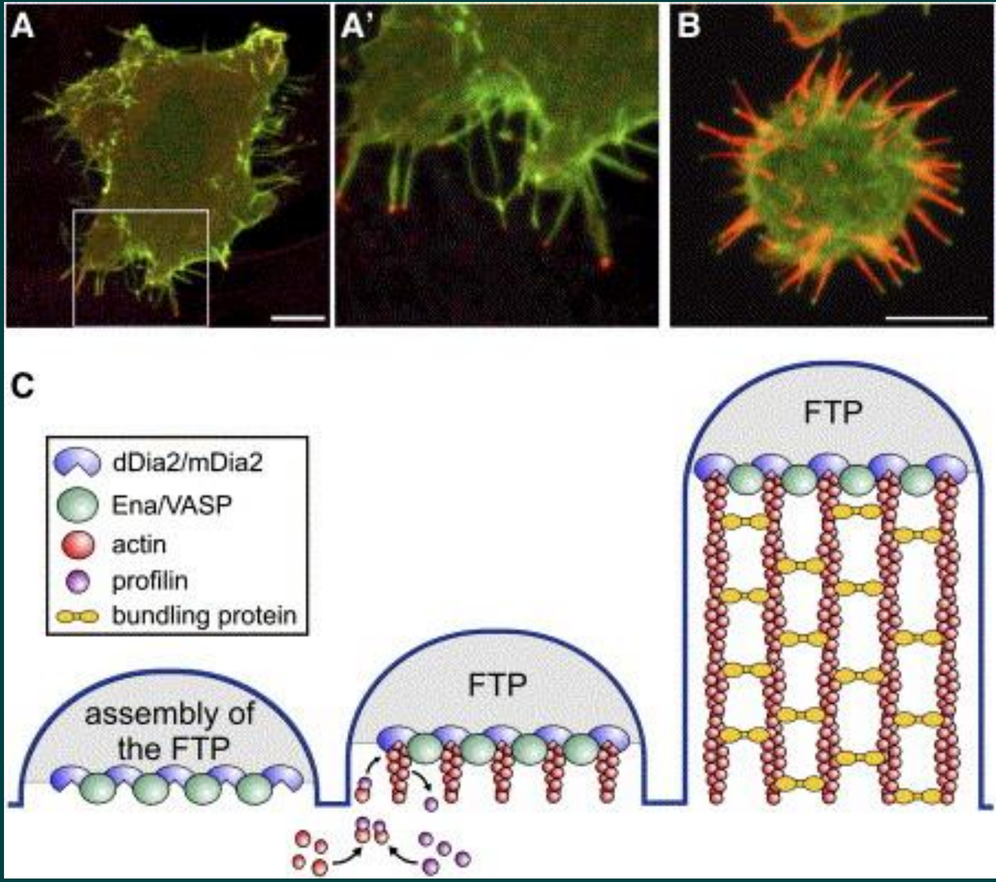
hypothesis on chirality arising from formin-mediated filament rotation



Tee et al., NCB, 2015

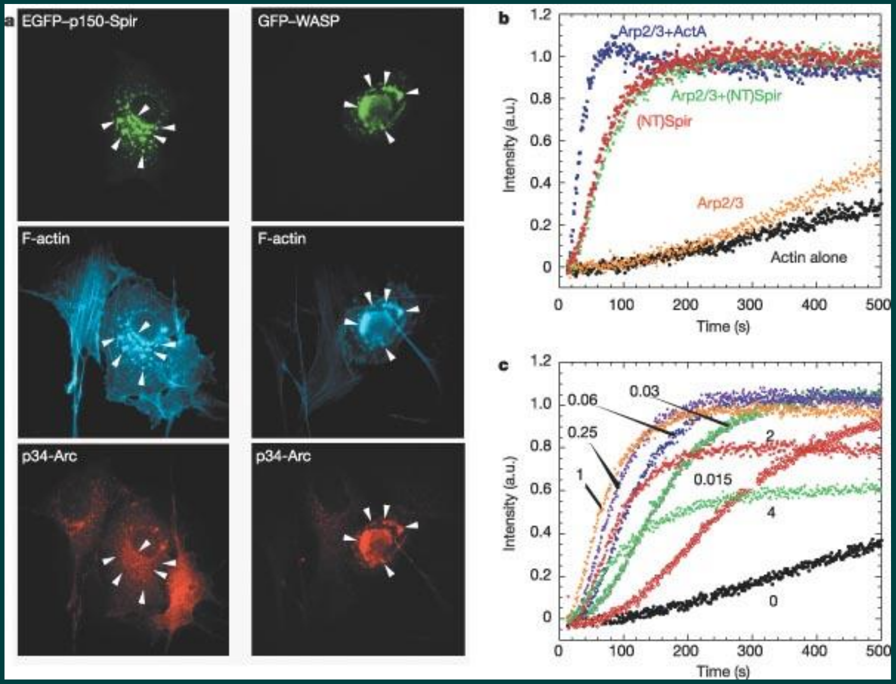
Role of formins in filopodia formation

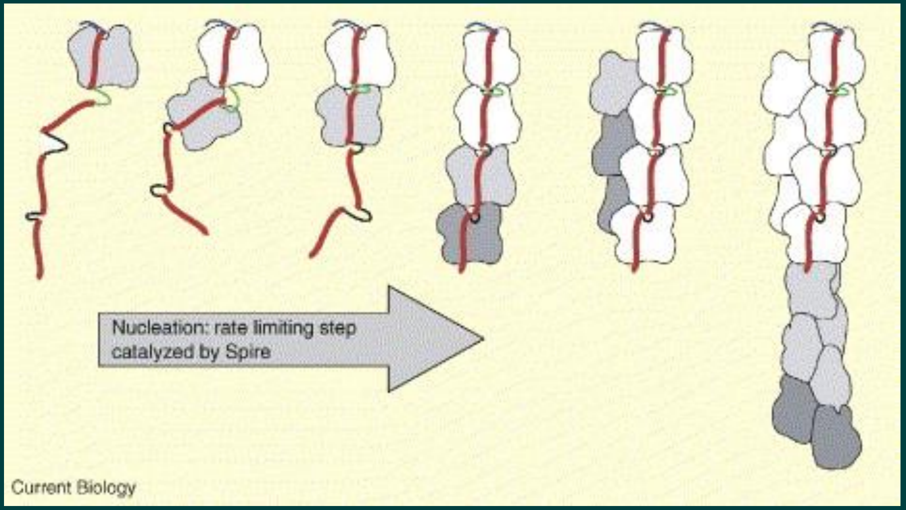




Schirenbeck et al., NCB, 2005

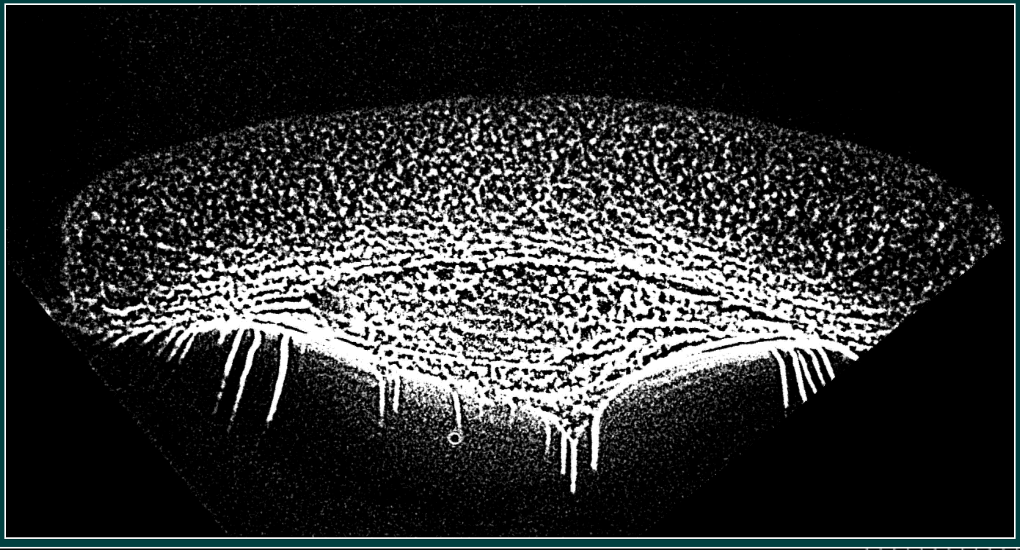
Third actin nucleator – Spire
(identified in Drosophilla, contains four WH2 domains)

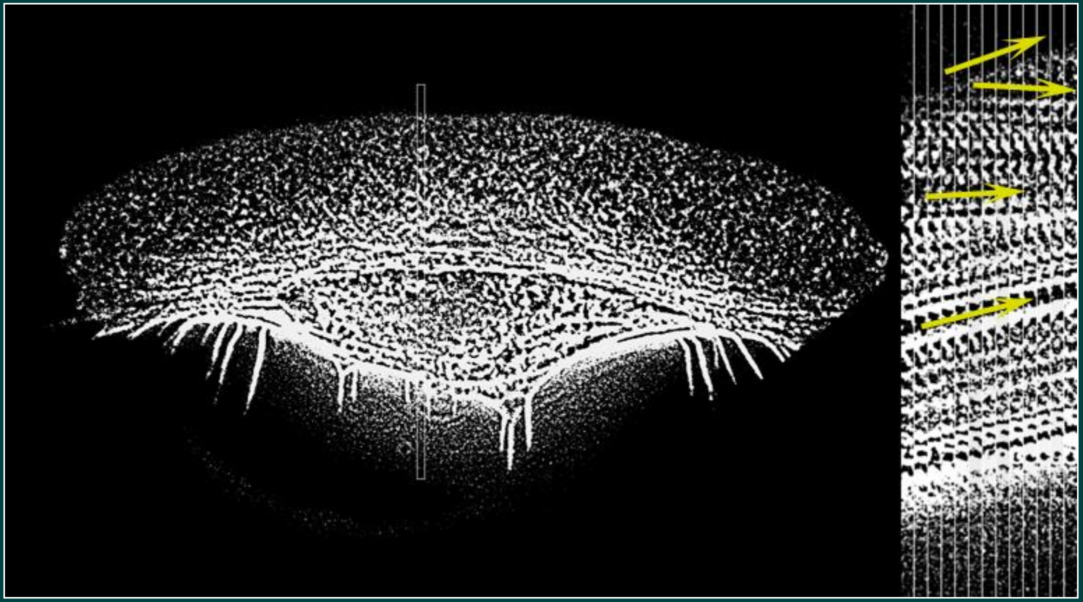




Quinlan et al., Nature, 2005
Baum and Kunda, Current Biol., 2005

polymerization is not enough:
assembly at the front, translocation at the back

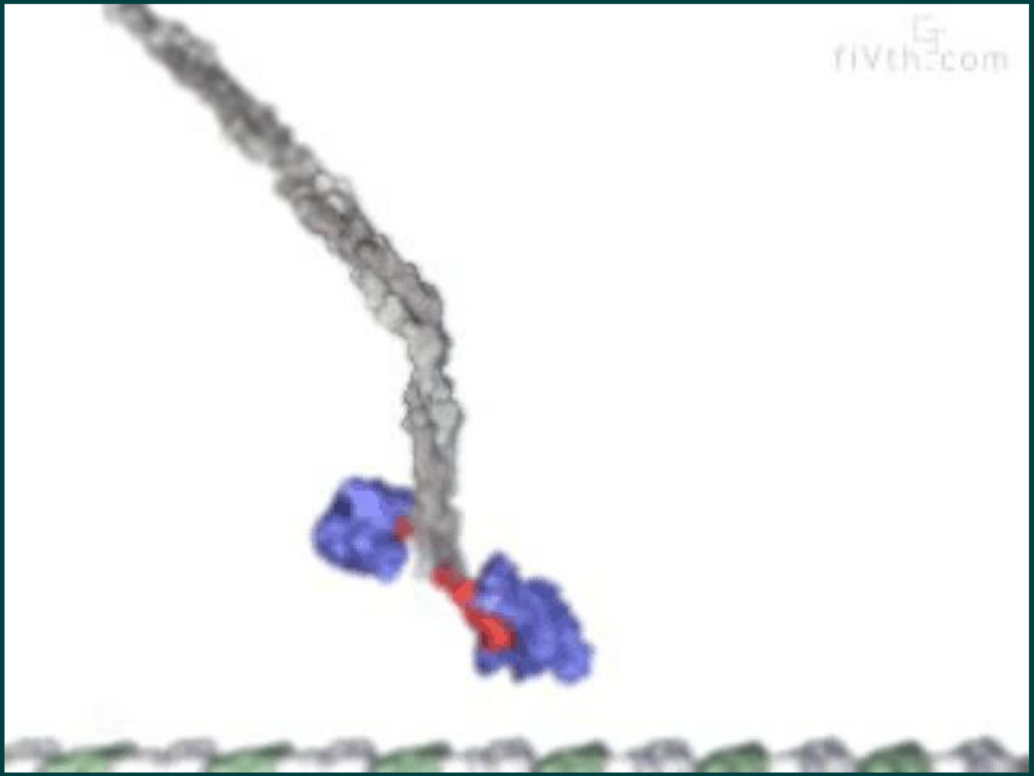




Schaub et al., MBC, 2007

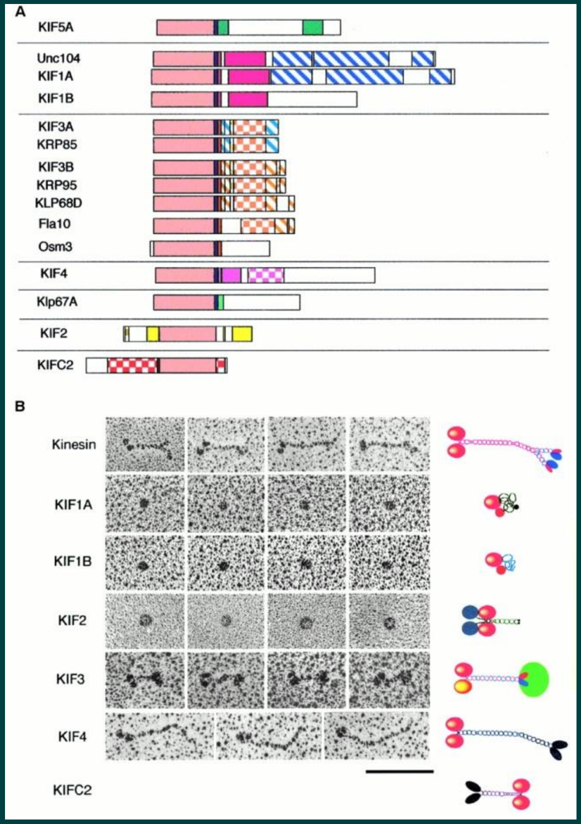
Is there an independent mechanism of force generation at the back?

Motor proteins

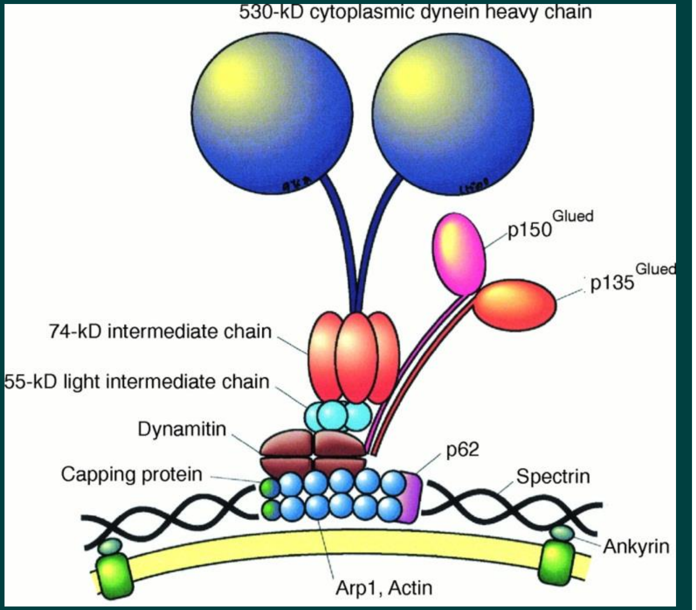


Cartoon of kinesin moving along a microtubule
(Vale lab website)

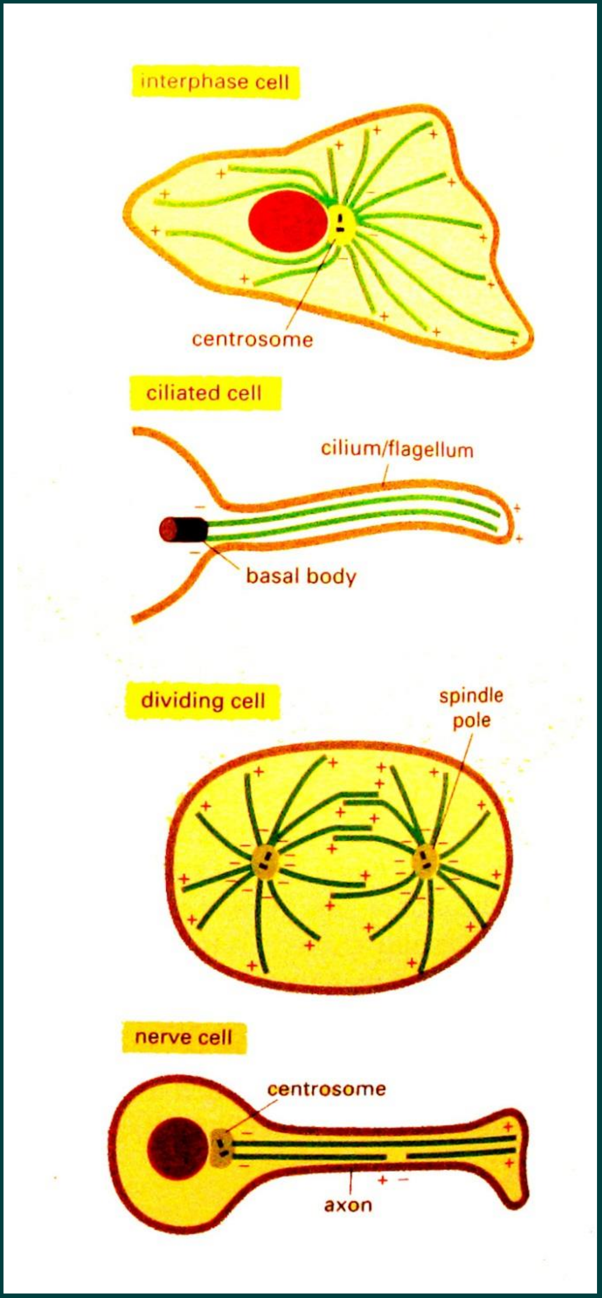
Microtubule-dependent motors



kinesins:
+ and – end directed

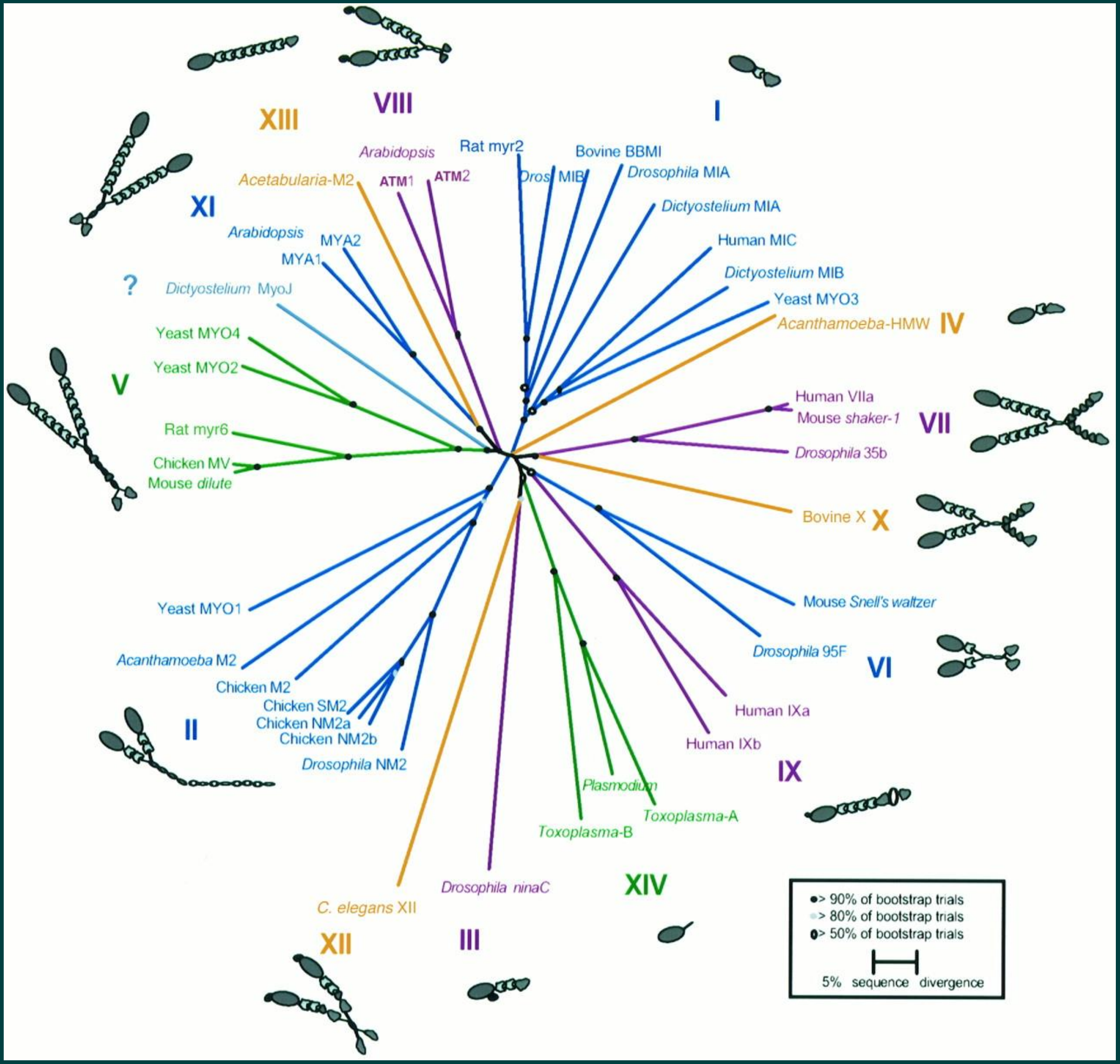


dyneins:
-end directed



microtubule polarity

Actin-dependent motors: myosins



myosin II in the muscle

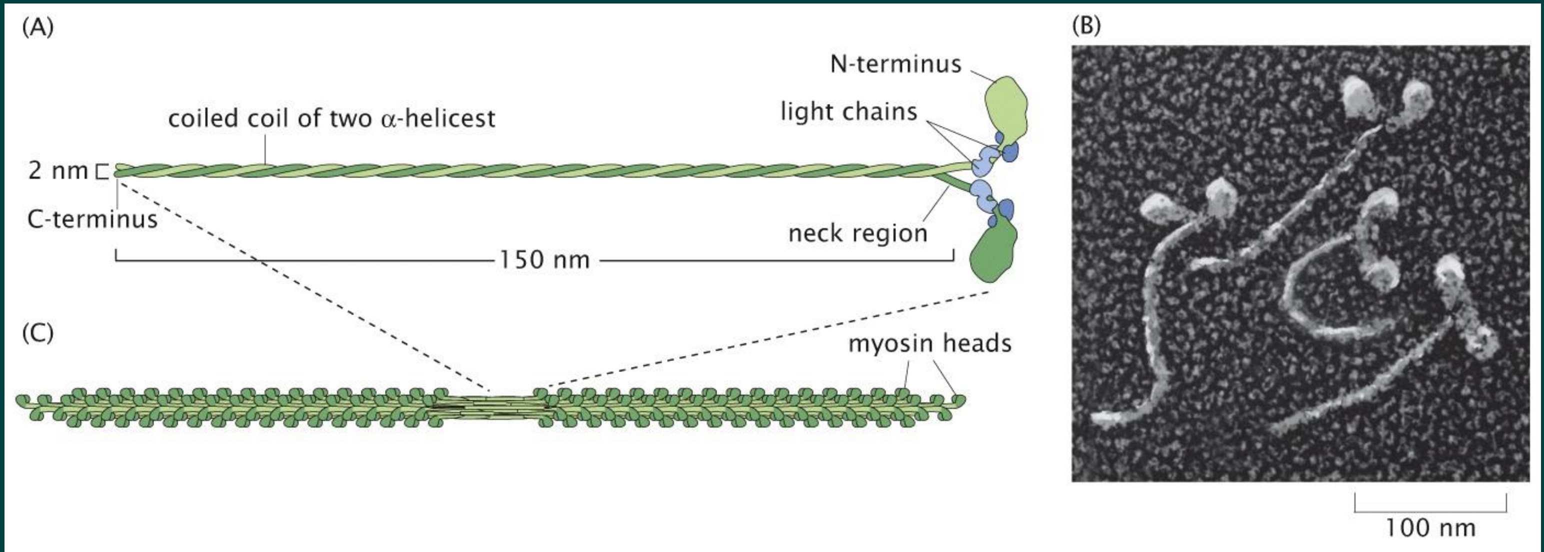


Figure 16.6 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

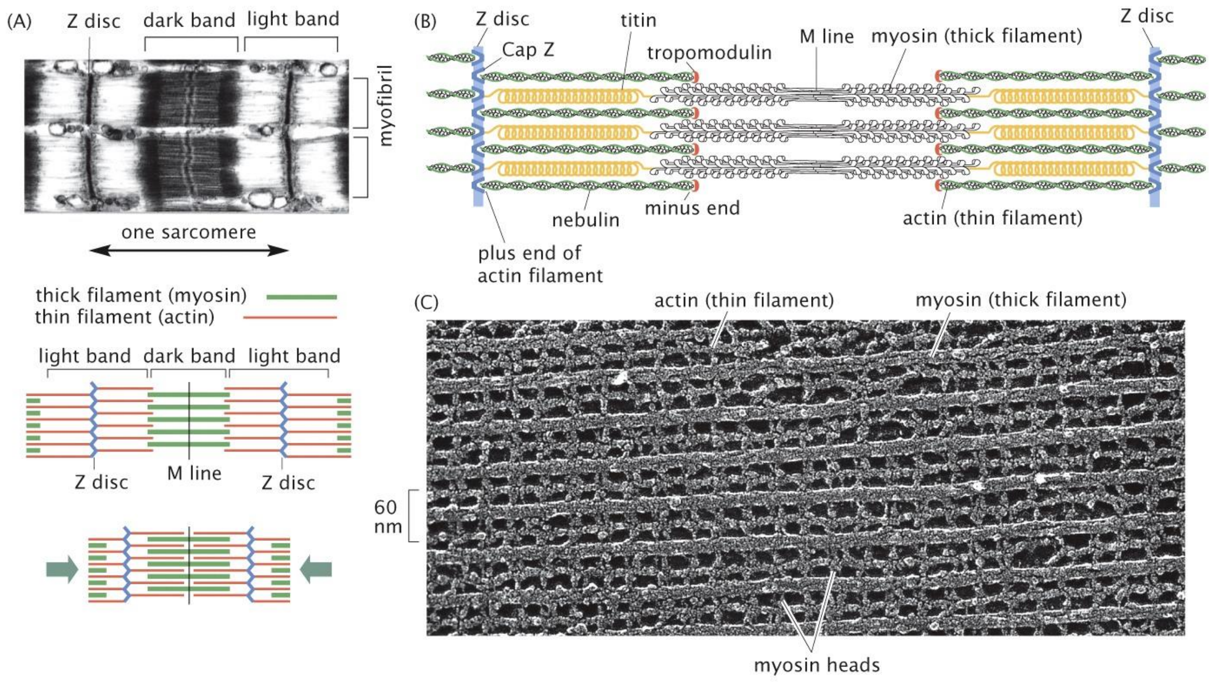


Figure 16.7 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

similar molecular designs in different motor proteins

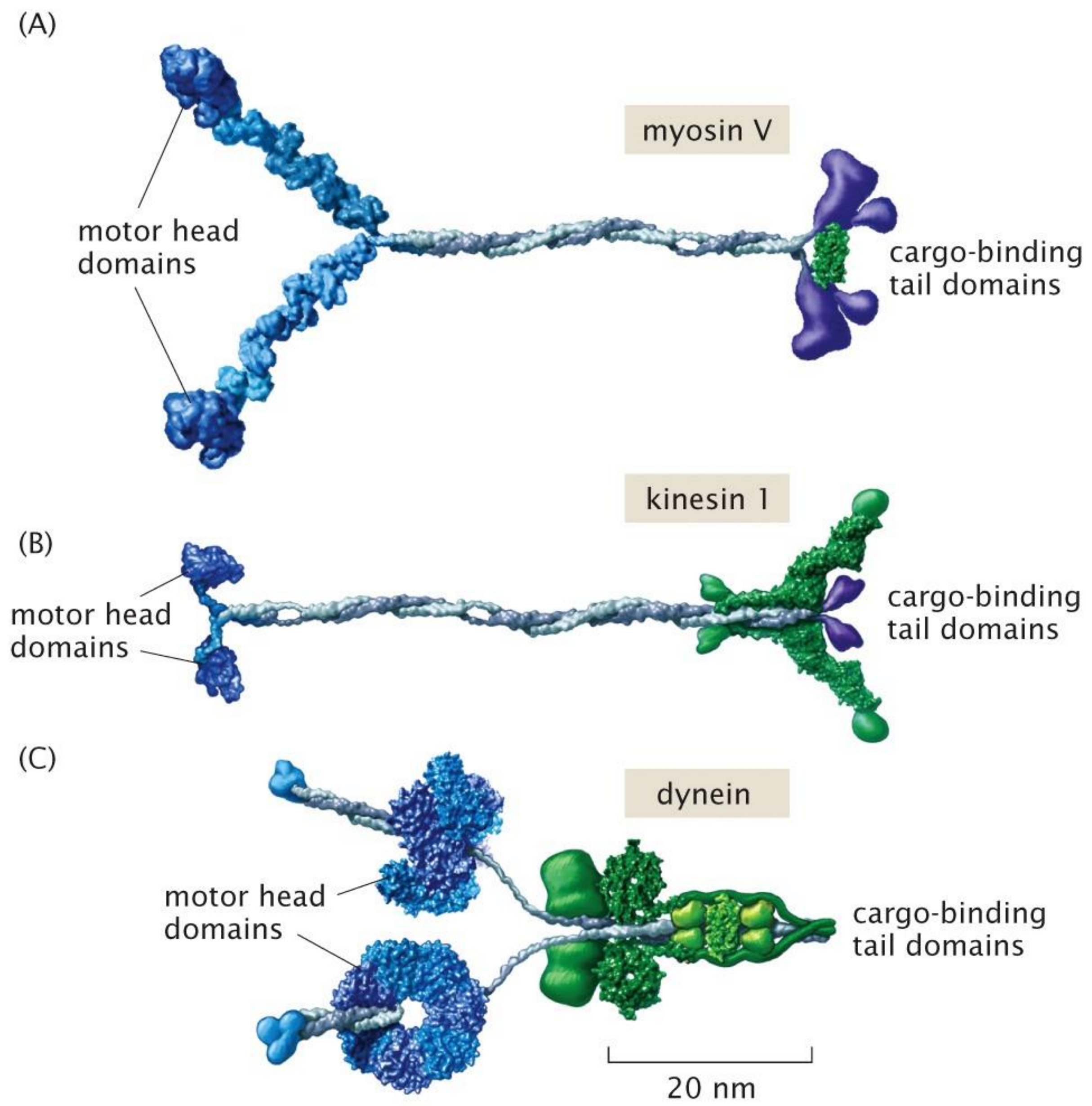


Figure 16.2 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

ways to study motors in action

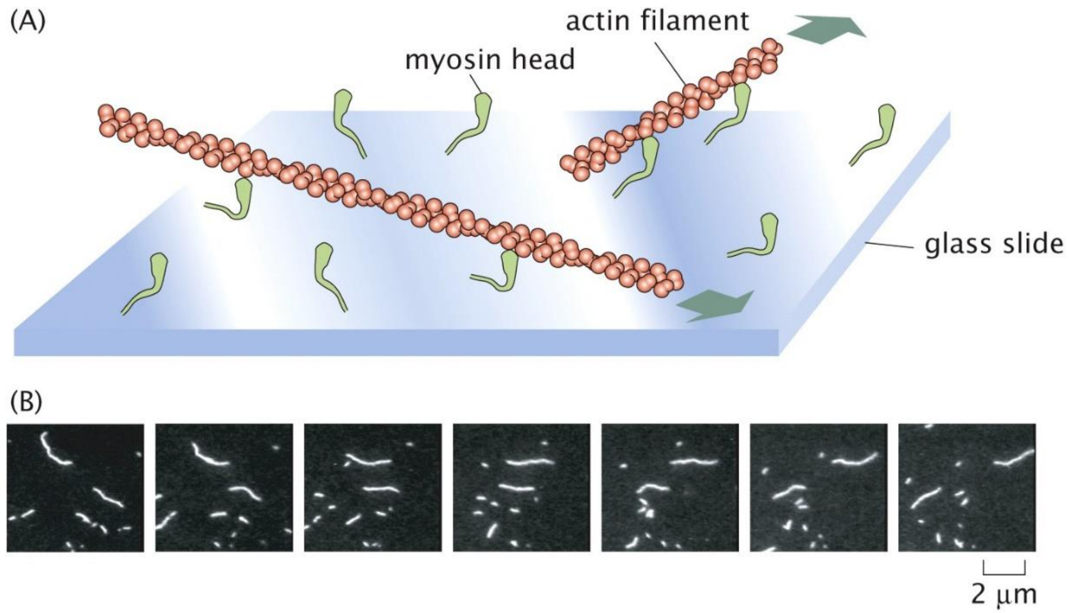


Figure 16.9 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

motors on the surface,
filaments in the solution

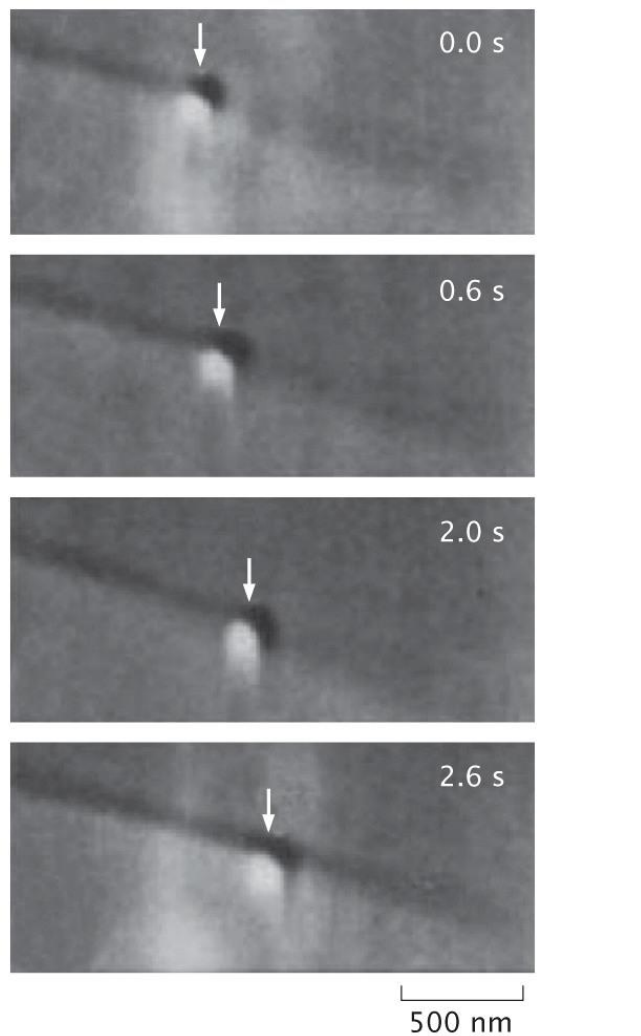
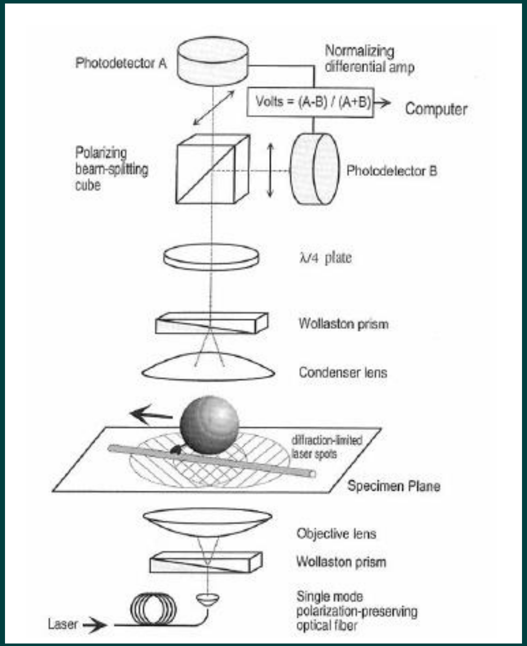
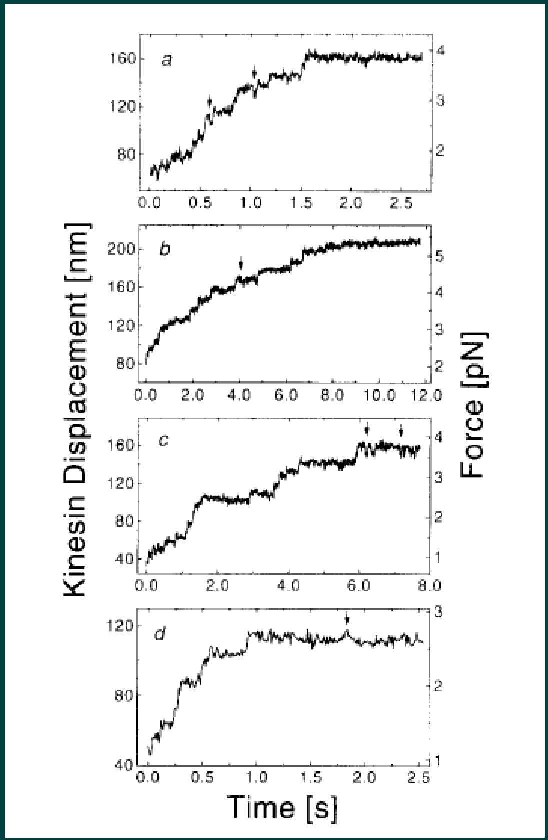


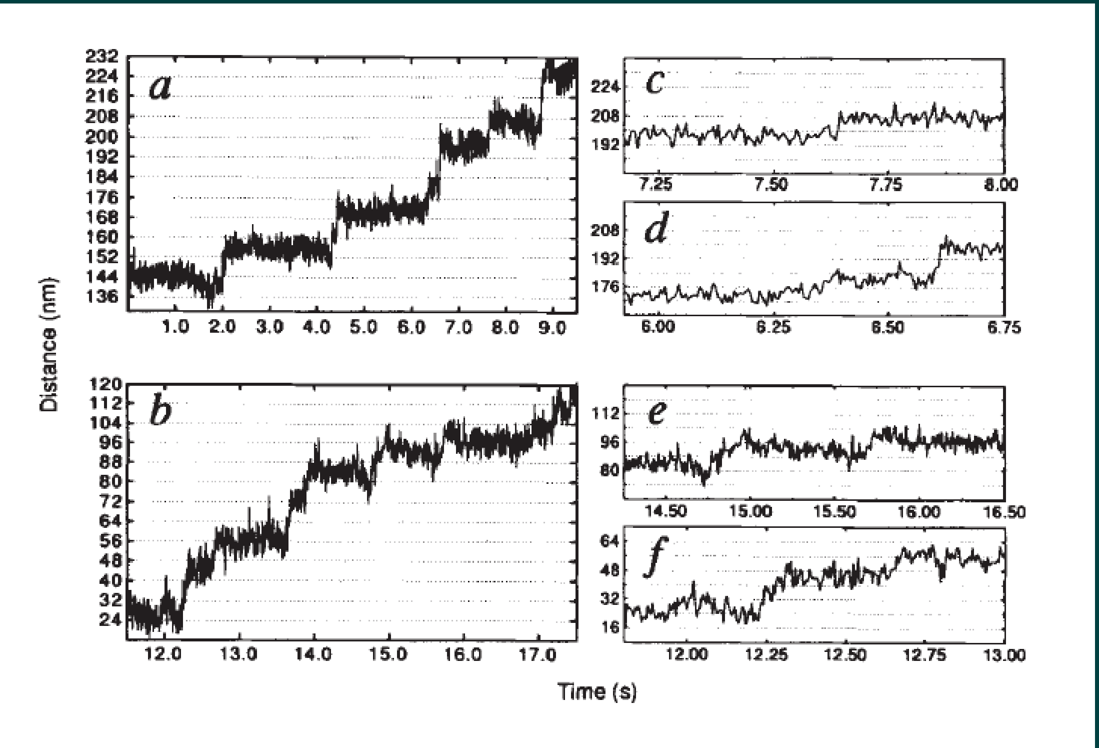
Figure 16.10 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

microtubule on the surface,
motors on a bead

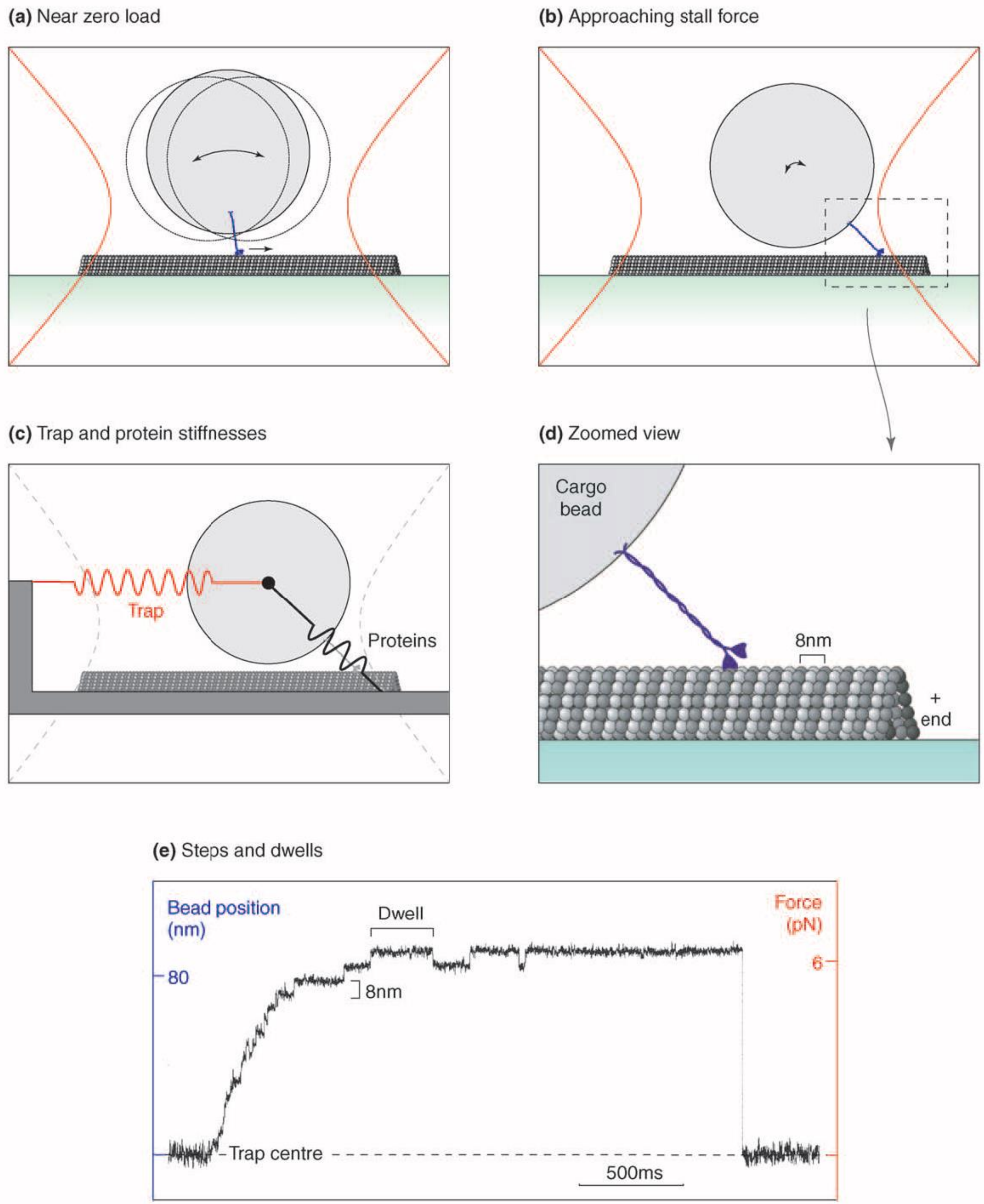


Kinesin's steps and forces





Svoboda et al., Nature, 1993
Svoboda and Block, Nature, 1994



Current Opinion in Cell Biology

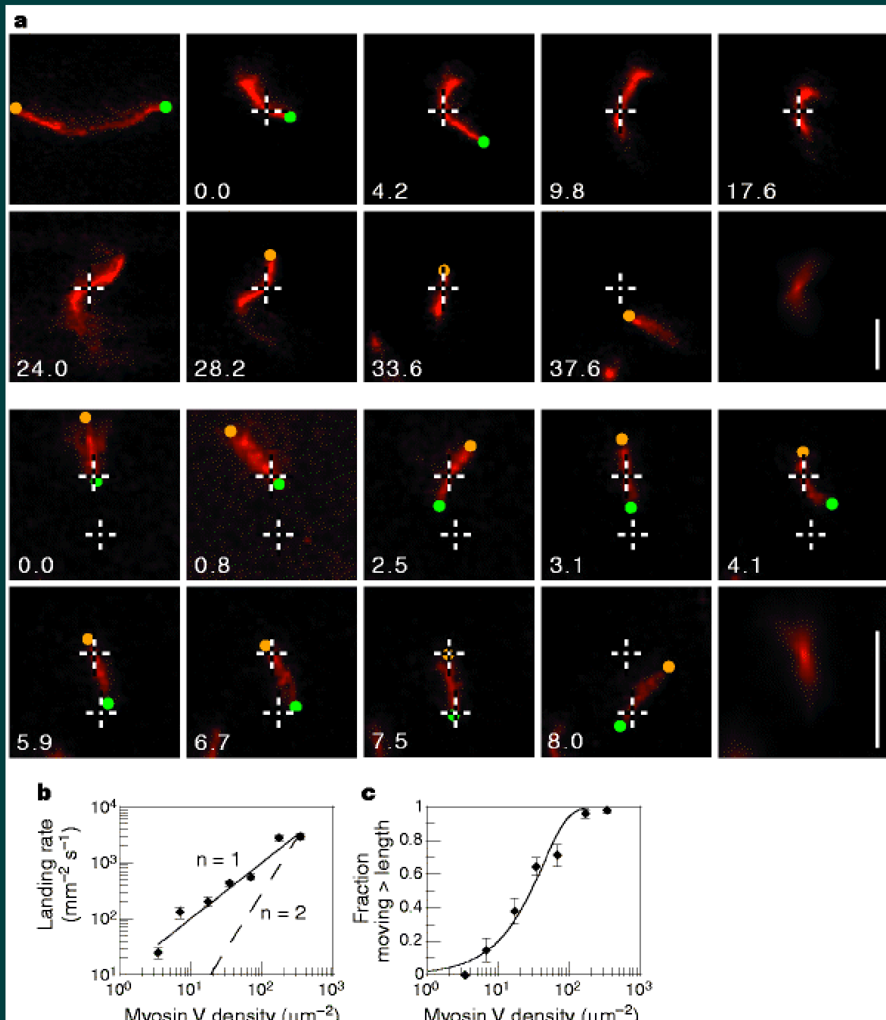
Biophysical properties of motors

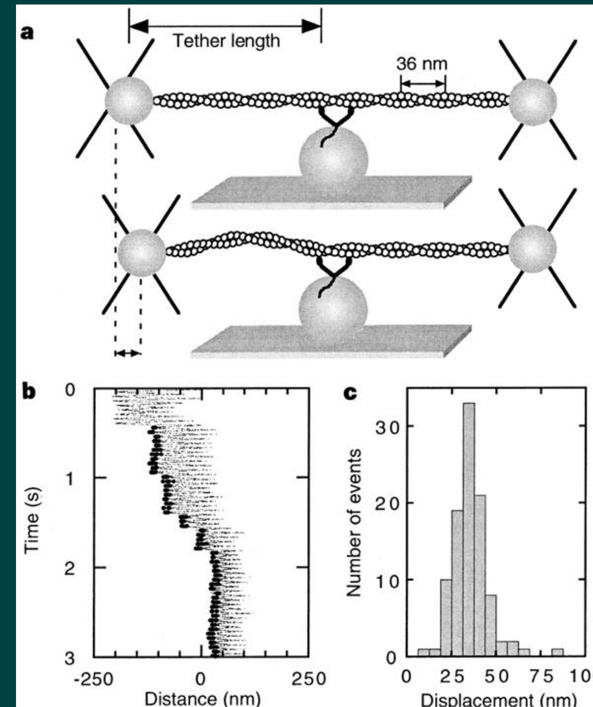
step size

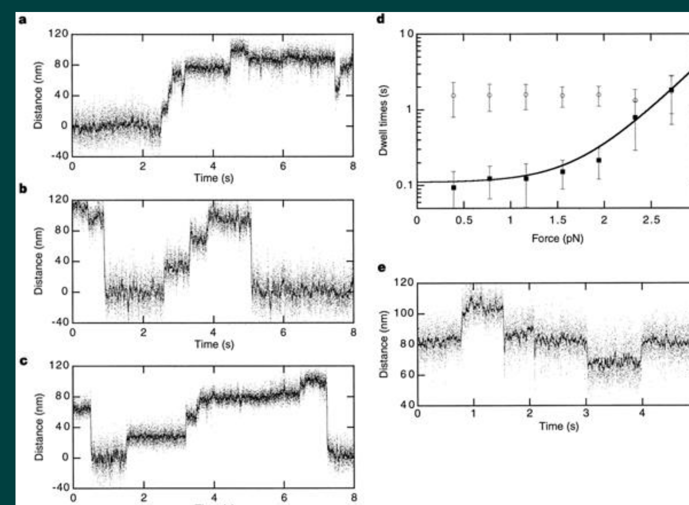
stall force

processivity: how many steps can motor take without detaching

Myosin V is a processive actin-based motor







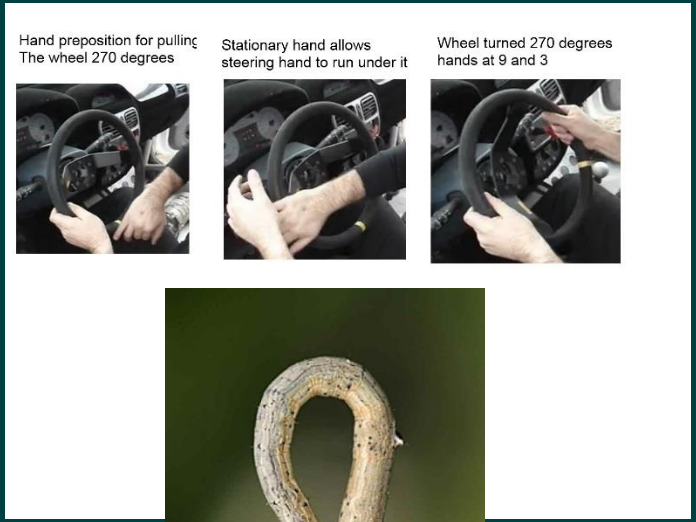
Mehta et al., Nature, 1999

What is the mechanism of processivity?

What is the mechanism of processivity?

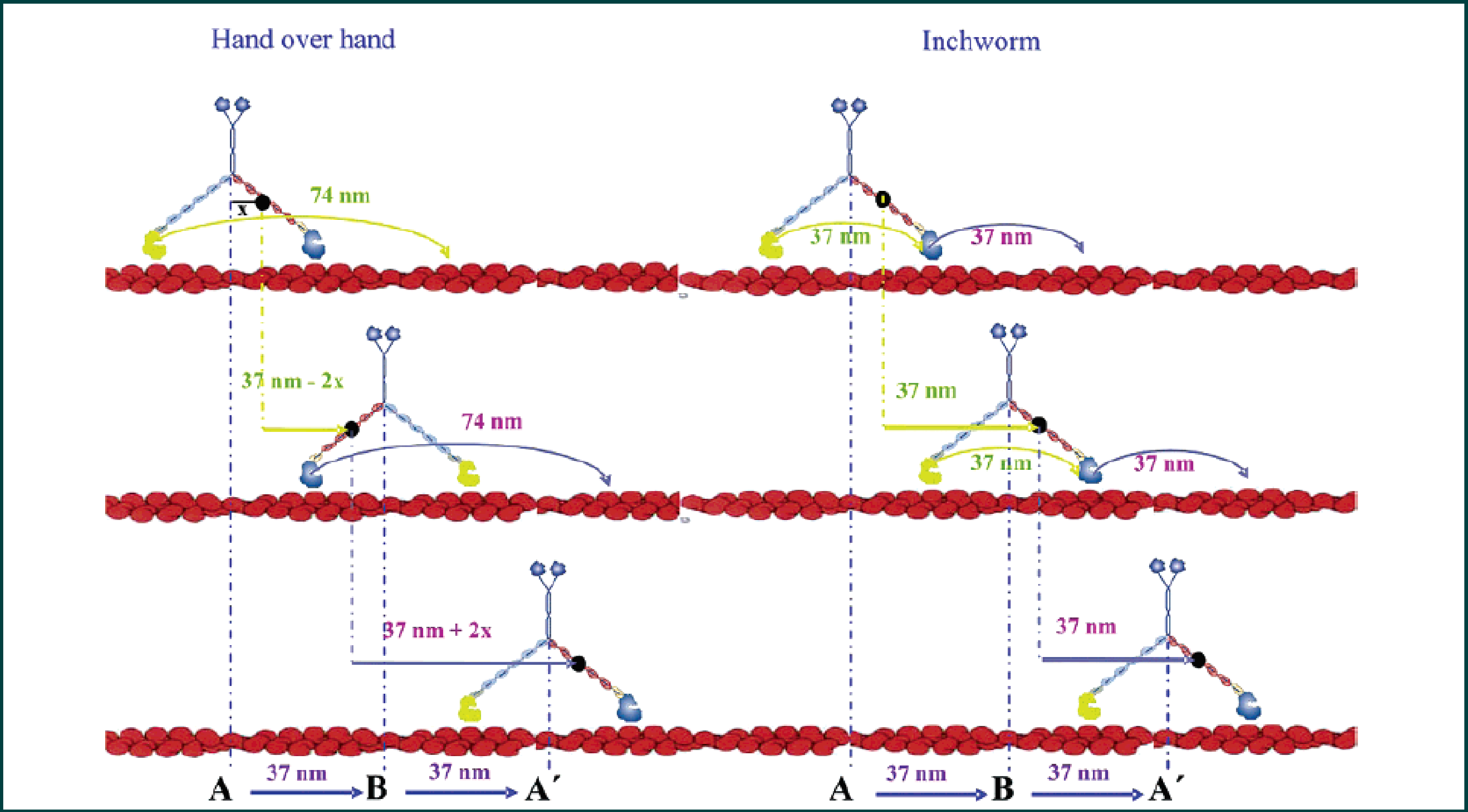
two active sites

hand over hand?

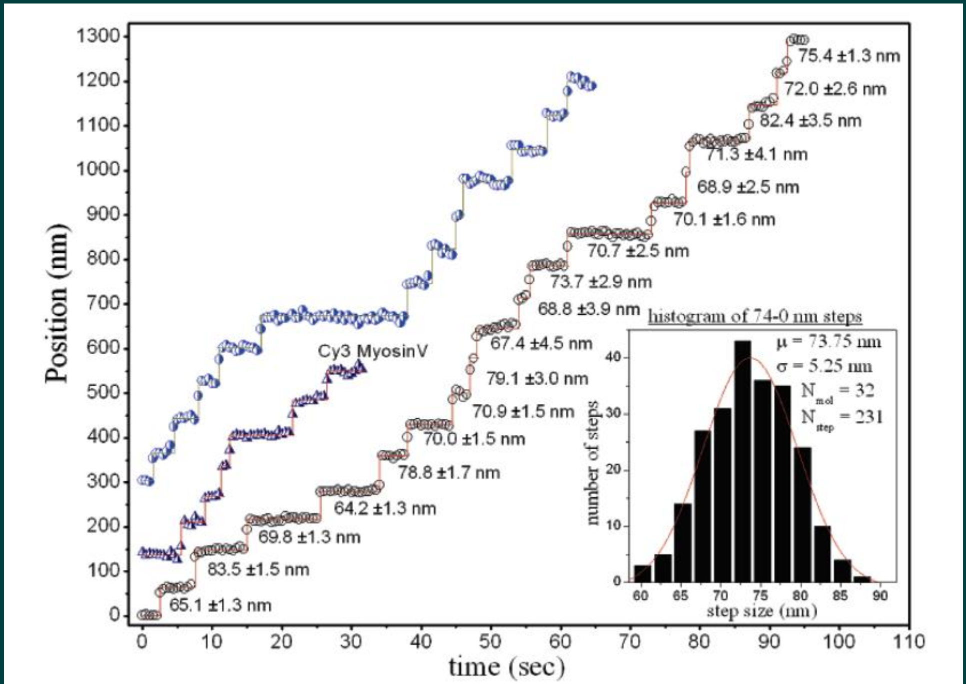


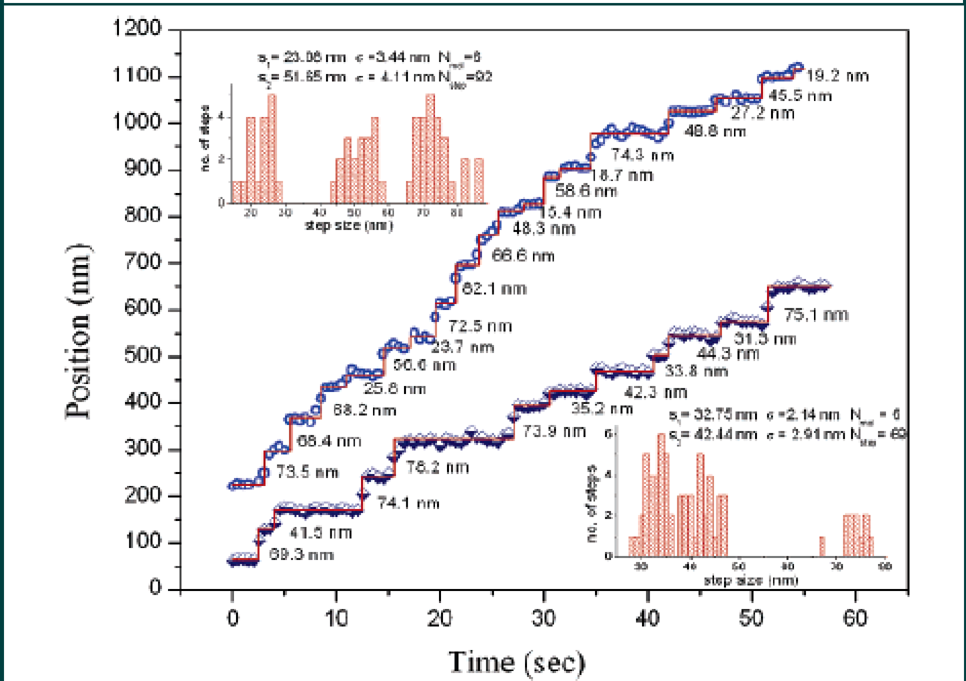
inchworm?

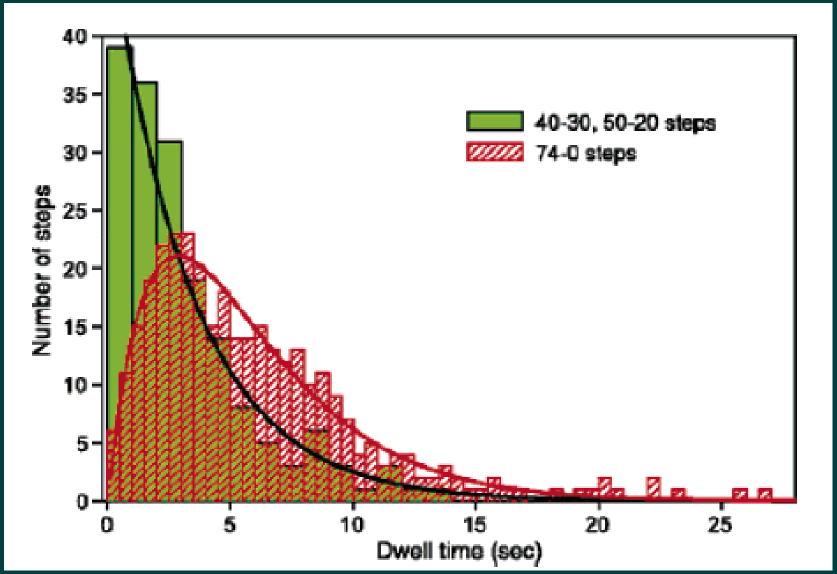
could be distinguished by labeling just one motor head in the dimer



Step sizes and dwell times for myosin V suggest hand over hand mechanism



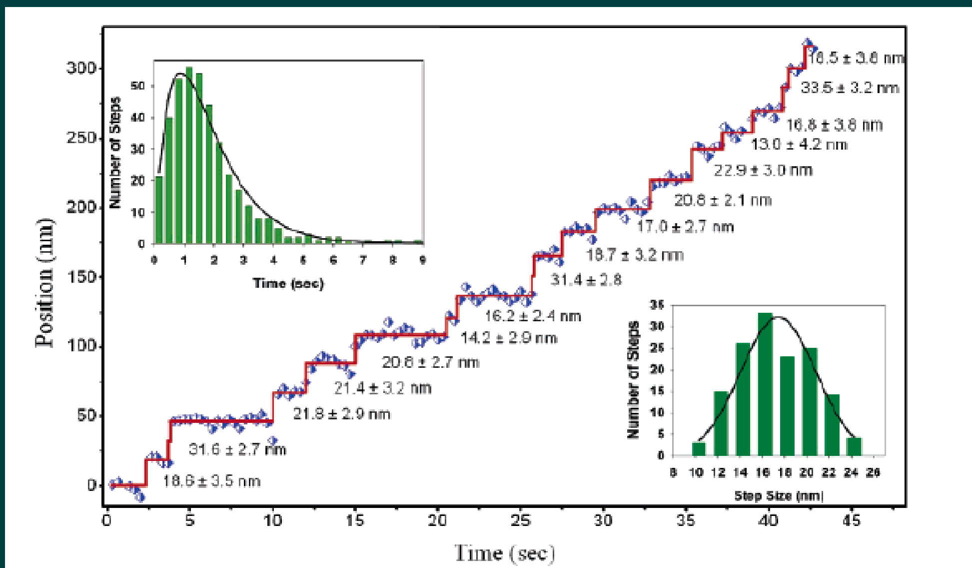


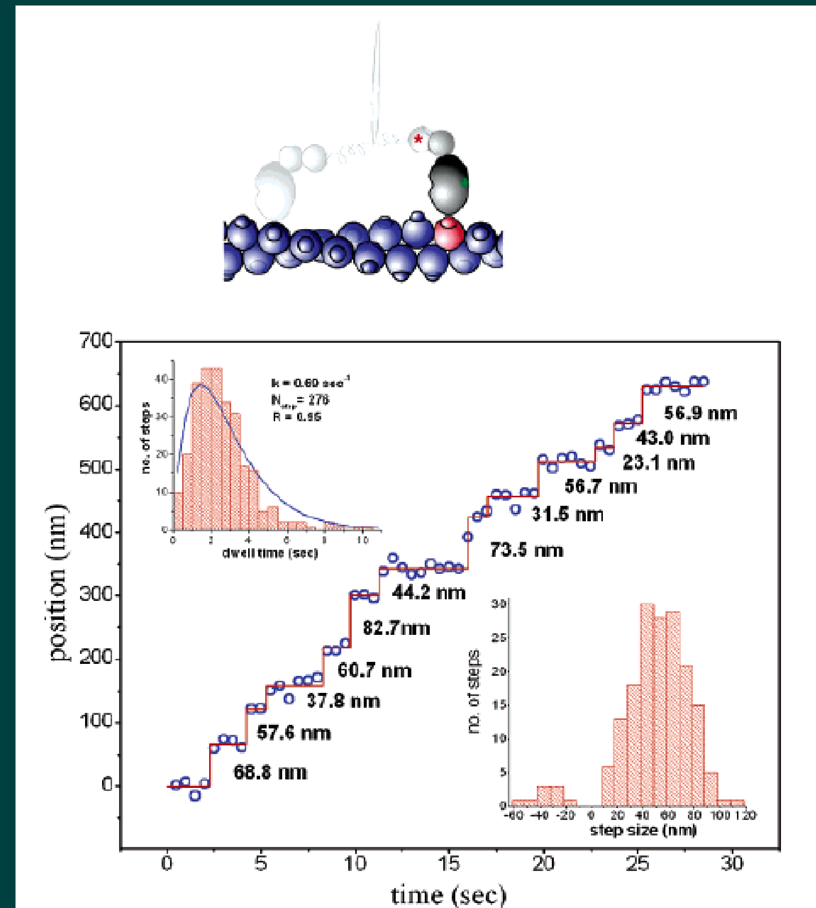


Yildiz and Selvin
Acc. Chem. Res., 2005

Myosin VI

Kinesin





Yildiz and Selvin, Acc. Chem. Res., 2005

Next time:

what if there are many motors on the same cargo?

what microtubule-dependent motors do in the cells
(transport, mitosis)