

# Actin Self-Assembly and Listeria Motility

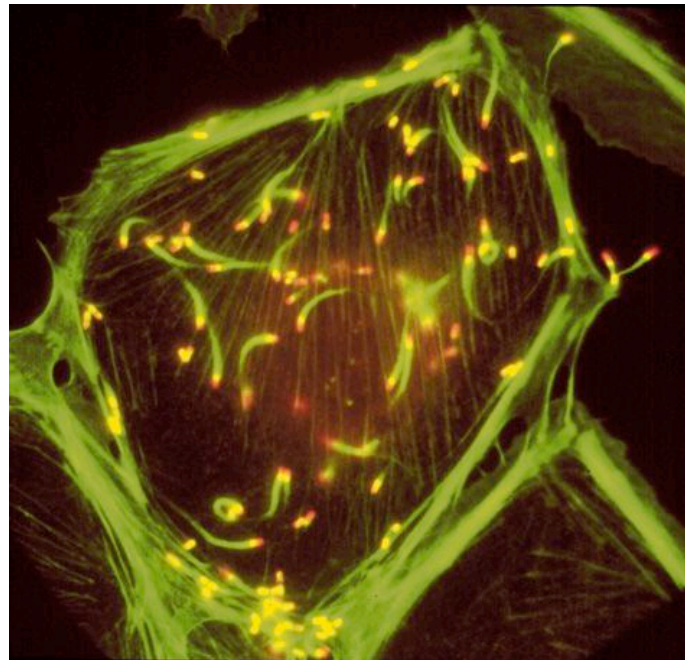
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University of Pennsylvania

Ajay Gopinathan  
Kun-Chun Lee

Physics & MRL, UCSB  
Physics & Astronomy, UPenn



# Cell Crawling

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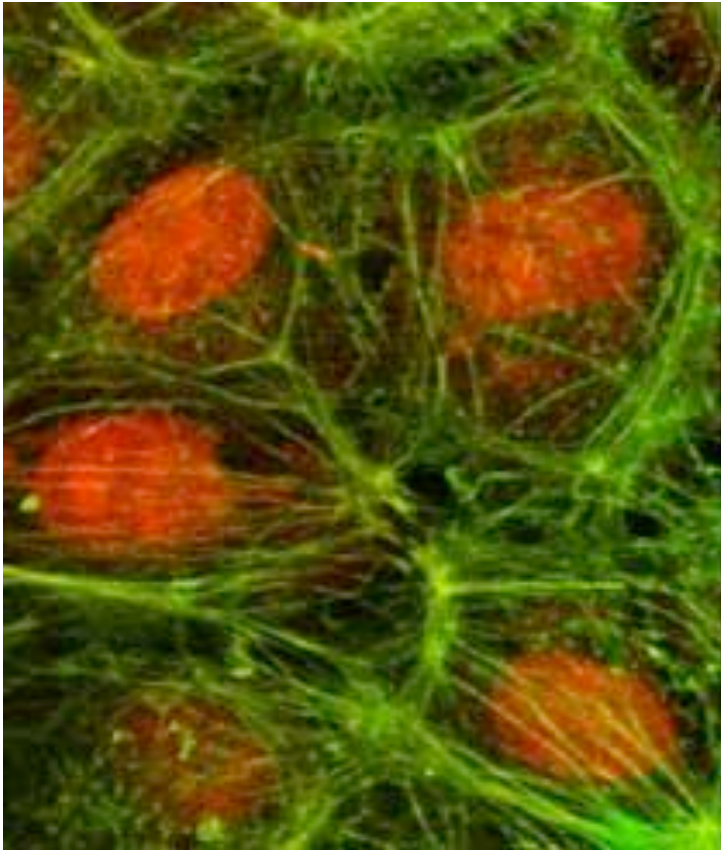
Neutrophil chasing *Staphylococcus aurea*

David Rogers, Vanderbilt University, 1959

[http://www.chem.uic.edu/fenteany/research/cell\\_migration/neutrophil.html](http://www.chem.uic.edu/fenteany/research/cell_migration/neutrophil.html)

# Cytoskeleton

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Courtesy of M. Gimbone

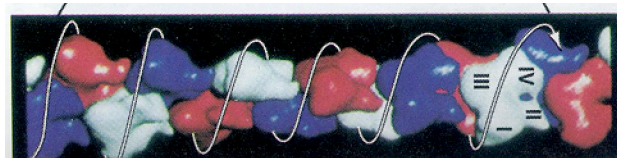
- Cytoskeleton gives cell its shape and mechanical rigidity
- It must reorganize when cells crawl
- Reorganization primarily due to **actin polymerization**

¿ How is polymerization converted into motion?

# Actin Polymerization and Depolymerization

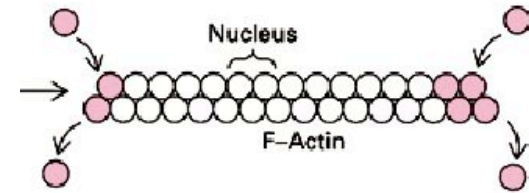


G-actin

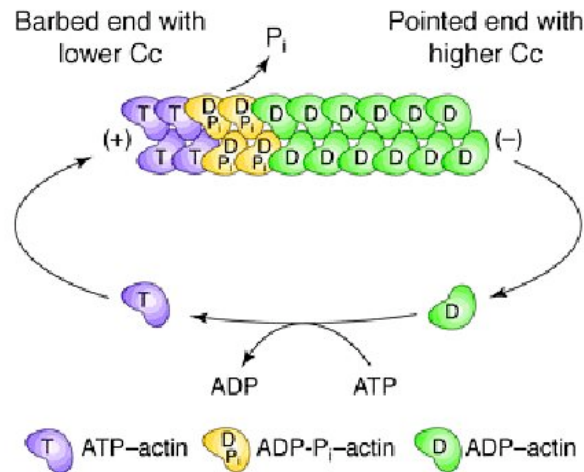


F-actin

Molecular Cell Biology, Lodish et al



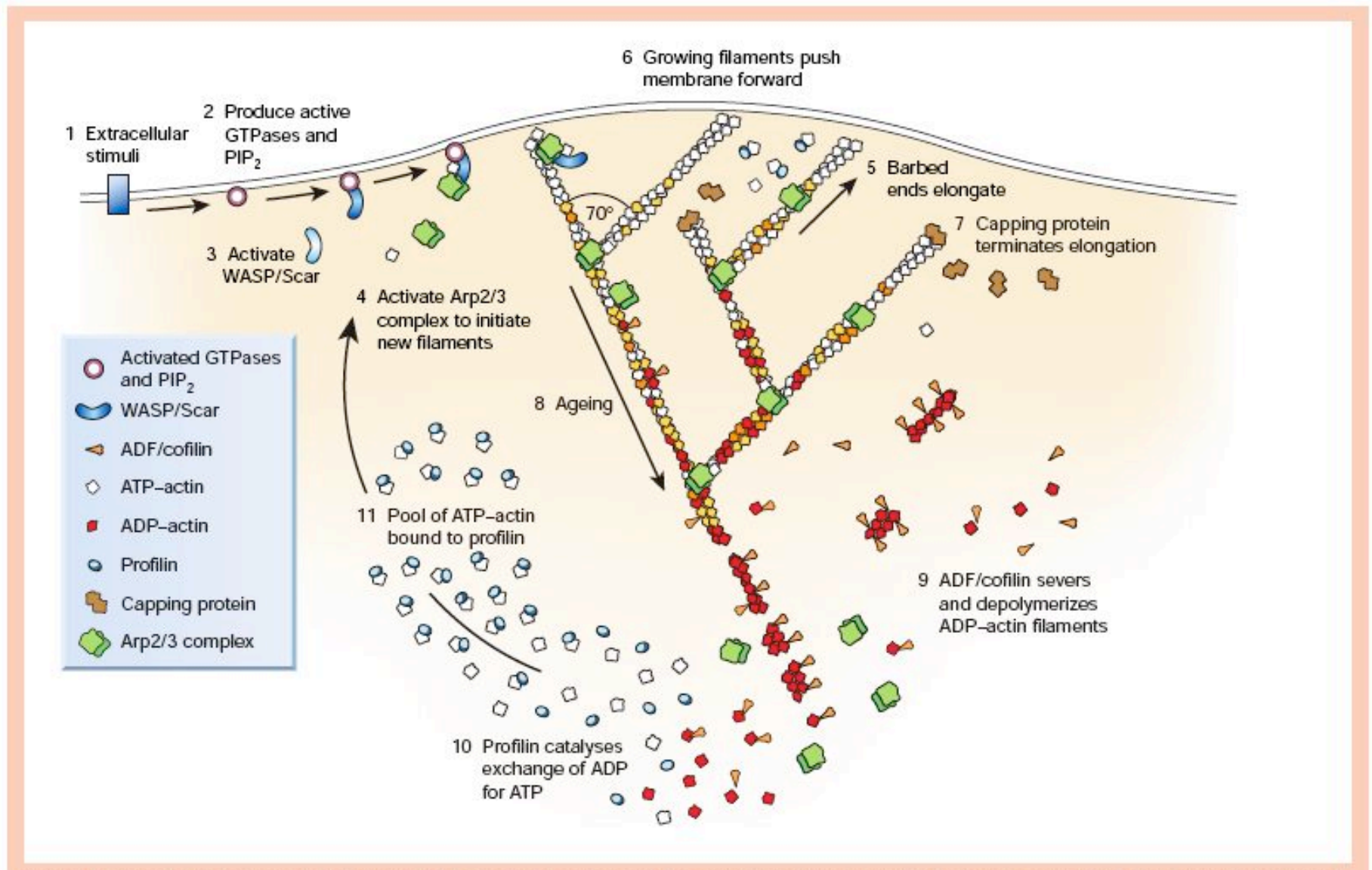
Steady state



From Chen, Bernstein and Bamberg,  
*Trends Biochem Sci* 2000 Jan., volume 25,  
pages 19-23.

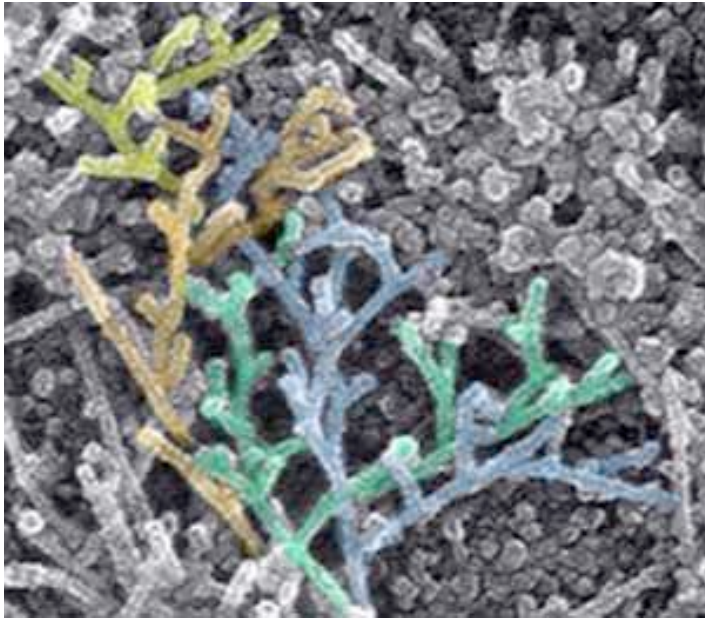
- A 3 $\mu$ m long filament turns over in 1 min *in vivo*
- ATP hydrolysis provides polarity to filament growth
- Growing ends localized near cell membrane

# Dendritic Nucleation Model: Participating Proteins



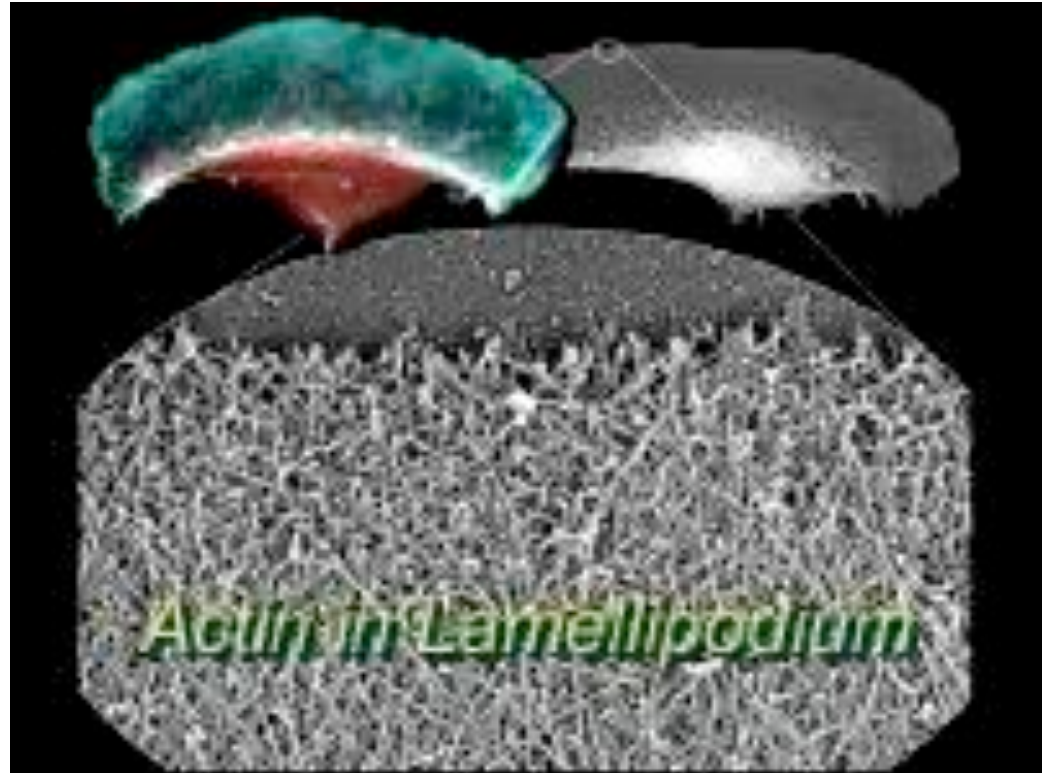
# Role of Branching

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T. M. Svitkina, G. G. Borisy,  
*J. Cell Biol.* **145**, 1009 (1999).

Arp2/3 complex binds to  
F-actin and nucleates  
new branches

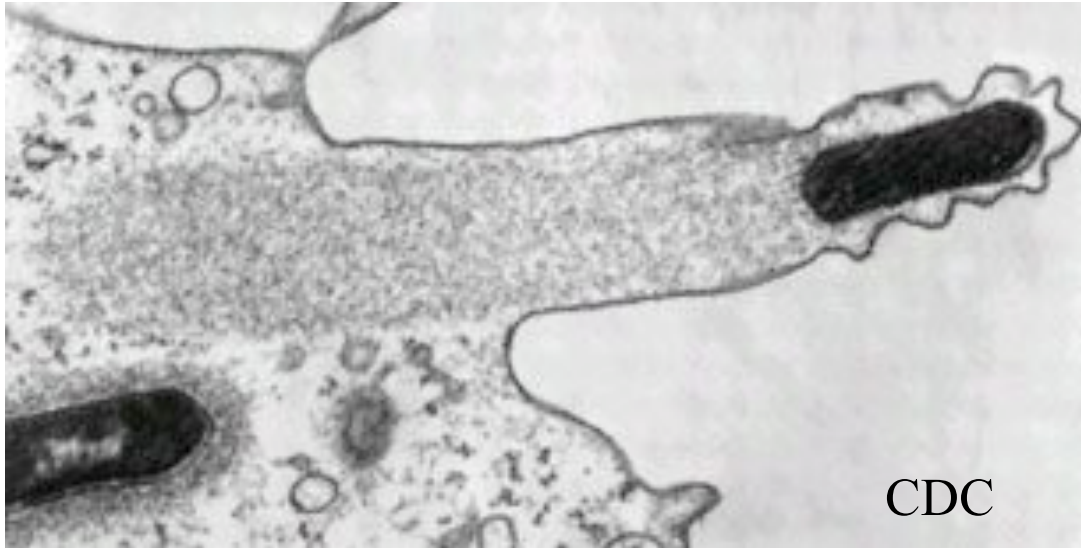


Courtesy: T. M. Svitkina, G.G. Borisy

[www.borisylab.northwestern.edu](http://www.borisylab.northwestern.edu)

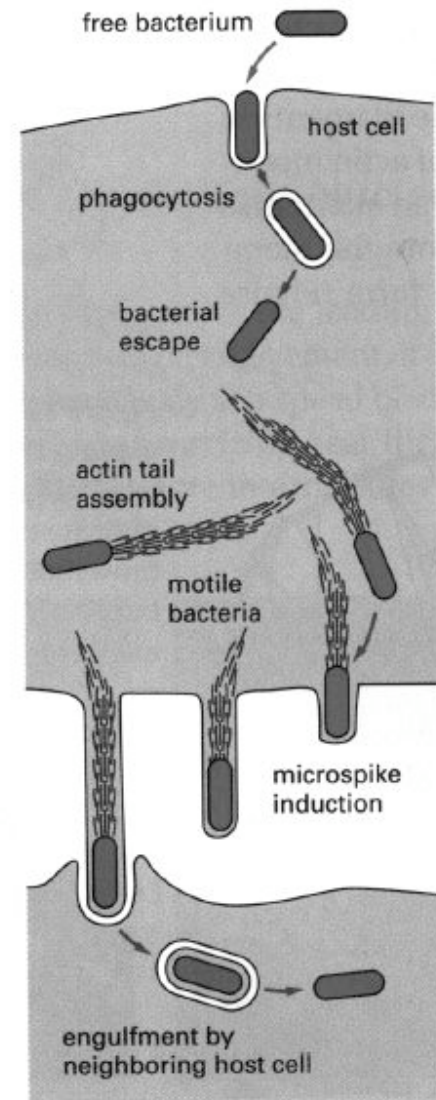
*Xenopus* keratocytes

# Actin and Listeria Motility



## Life cycle of *Listeria monocytogenes*

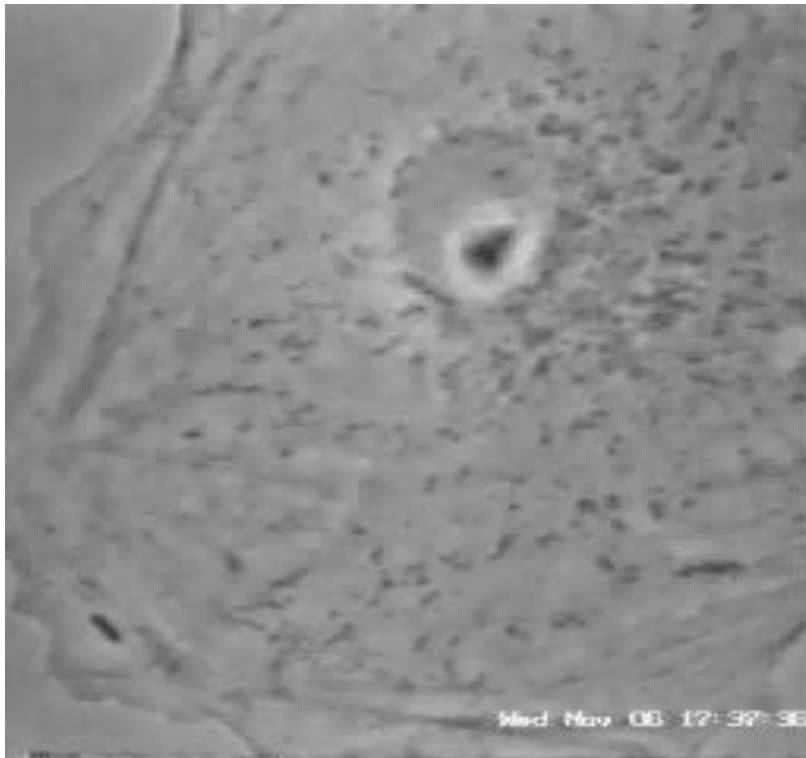
Uses actin polymerization to move!  
Same physics as cell crawling



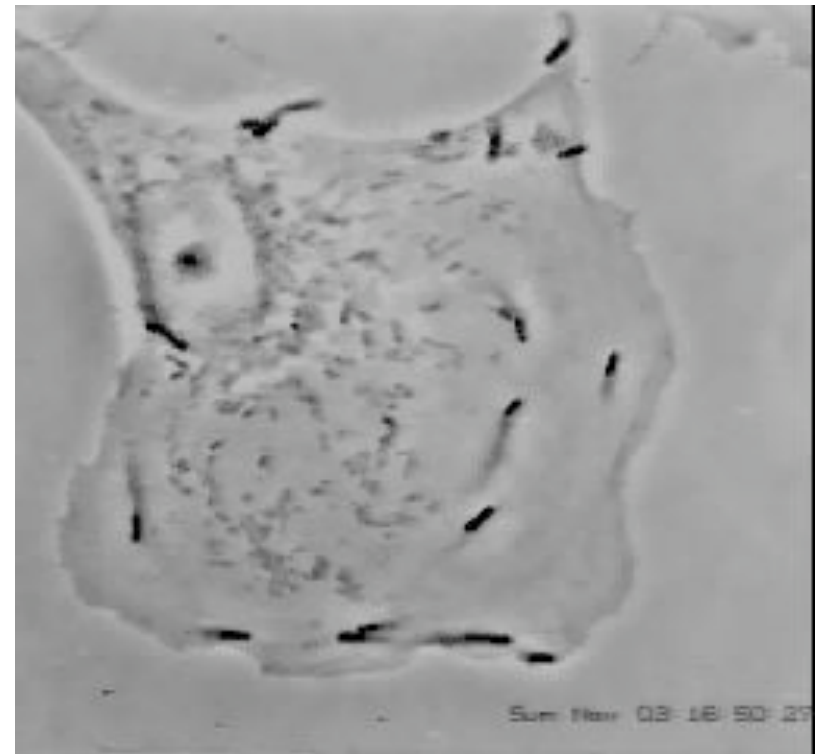
# How Listeria Spreads from Cell to Cell

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x900



x150



Courtesy of Julie Theriot, <http://cmgm.stanford.edu/theriot/>

Without proteins that generate comet tail, Listeria can divide but cannot spread to other cells

Comet tail is nearly **stationary**



# Polystyrene Beads Can Do It, Too!

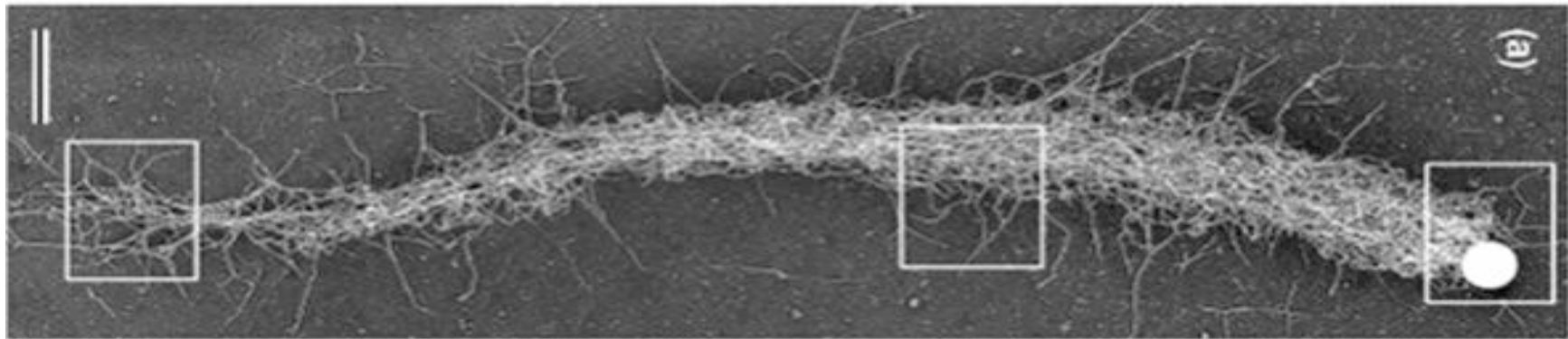
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Courtesy of Julie Theriot,  
<http://cmgm.stanford.edu/theriot/>

x60

Actin comet tail has same  
branchy structure as in  
lamellipodium



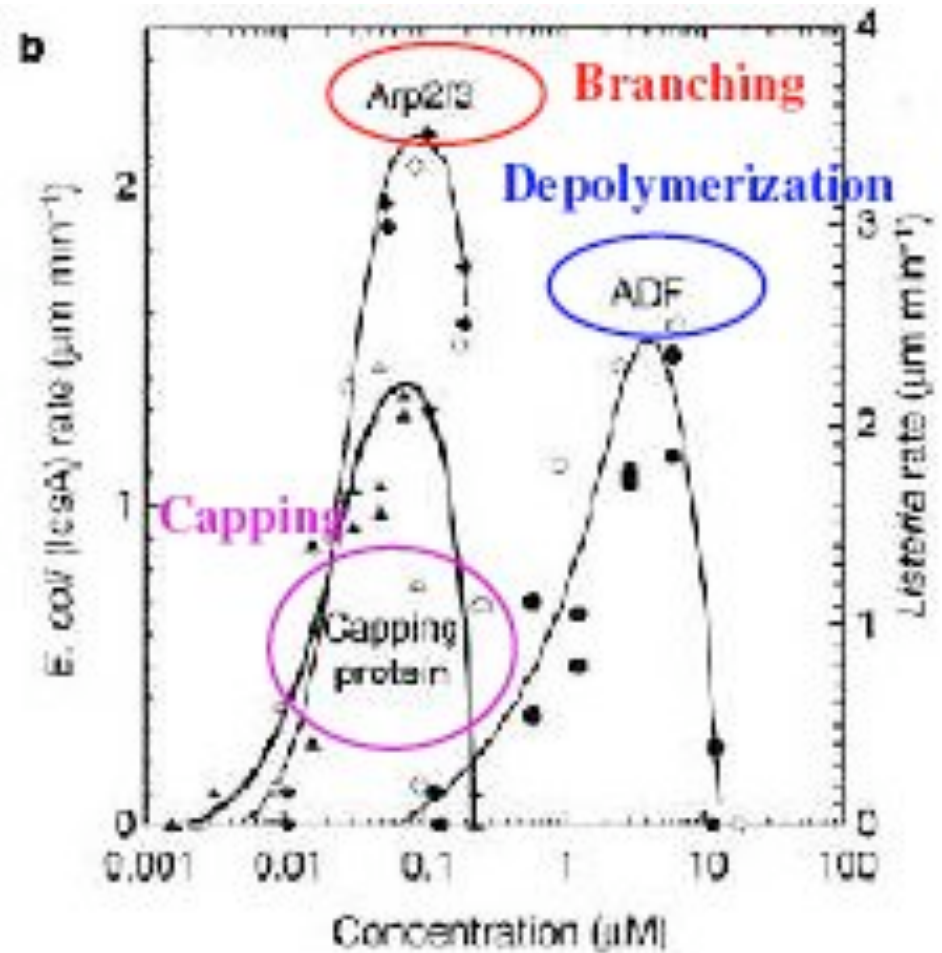
L. A. Cameron, T. M. Svitkina, D. Vignjevic, J. A. Theriot, G. G. Borisy,  
*Current Biology*, **11**, 130 (2001).

# Minimal Ingredients for Motility

"All" you need is

- Actin and buffer w/ATP
- Arp2/3 makes new growing ends
- Capping protein kills them off
- ADF/cofilin severs filaments
- Profilin converts ADP-G-actin to ATP-G-actin
- Bead coated with ActA,VCA, activates arp2/3

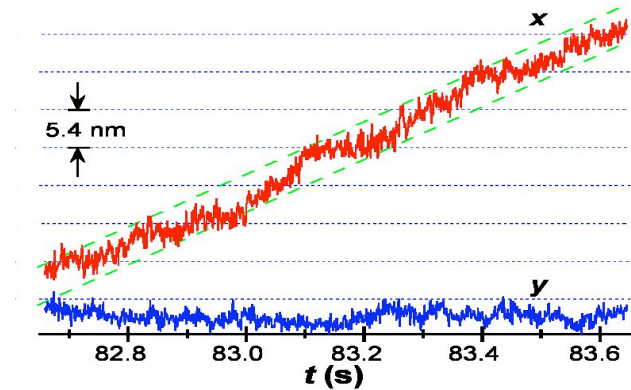
Loisel, Boujemaa, Pantaloni, Carlier,  
Nature, 401, 613 (1999).



# Signatures of the motility

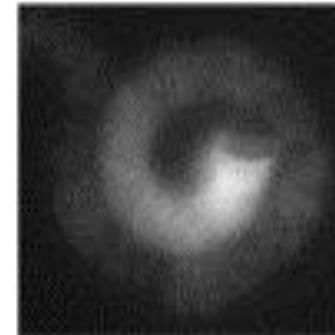
- Nanoscale displacement fluctuations

S.C. Kuo and J.L. McGrath, *Nature* 407, 1026 (2000)



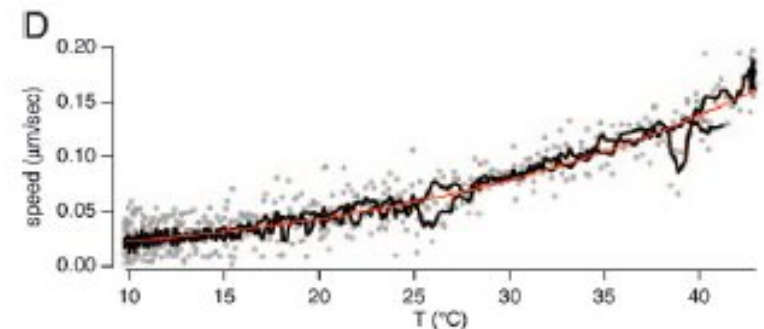
- Propulsion of flat discs as well as curved objects

I. M. Schwarz, et al, *Curr. Biol.* 19, 236 (2004)



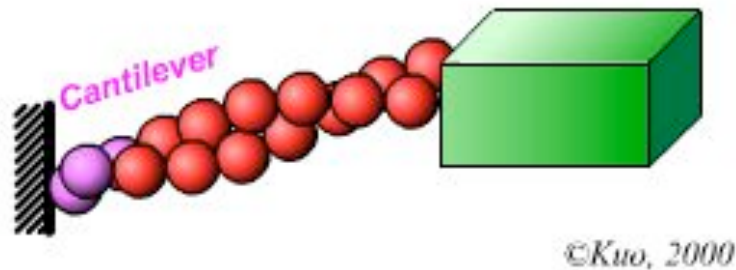
- Adhesion controls motility

F.Soo and J.A. Theriot, *PNAS*, 102, 45, 16233 (2005)



# Some Previous Models

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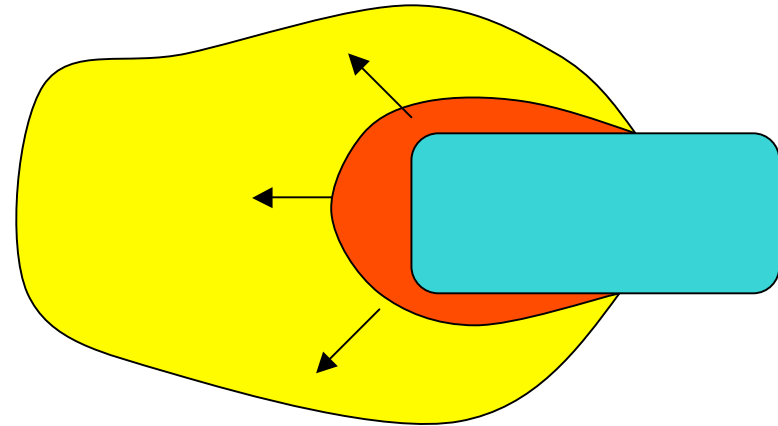


## Single Filament models A.

Mogilner and G. Oster, *Biophys. J.* 71, 3030 (1996); *Biophys J.* 84, 1591 (2003).

Working vs. attached filaments

**BUT** these are coupled together via actin gel structure



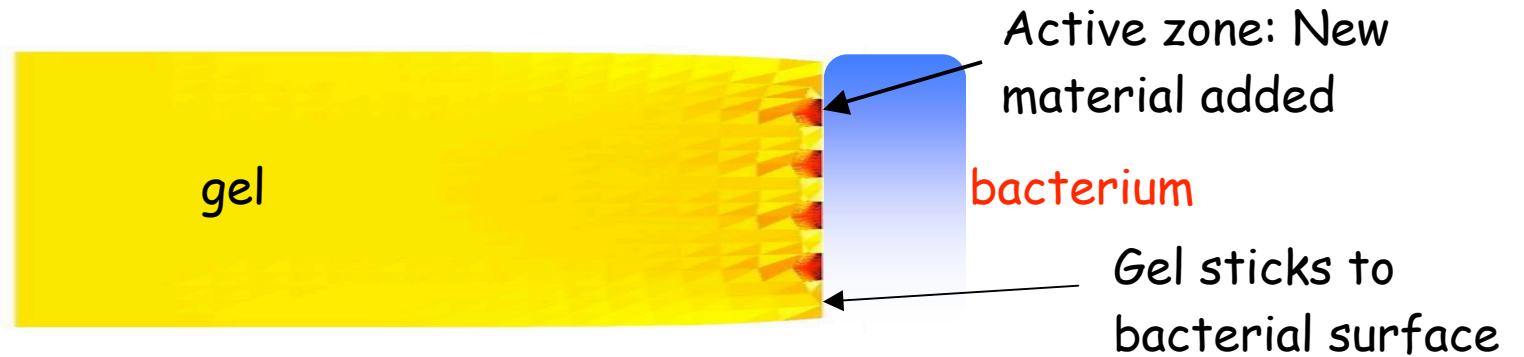
## Macroscopic models

F. Gerbal, P. Chaikin, Y. Rabin and J. Prost, *Biophys. J.* 79, 2259 (2000)

**BUT** predicts no motility for flat disks; no nanoscale fluctuations

# Our Model (Ajay Gopinathan)

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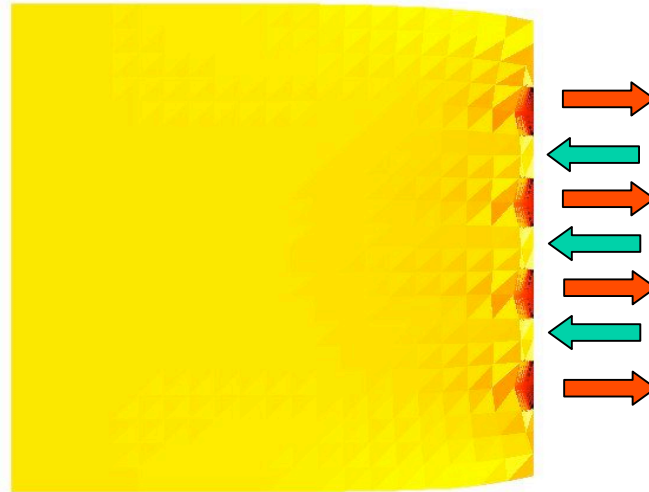


- New material is added at active zones
- Gel **tethered** w/ binding energy  $E_b$  can unbind; gaps filled with new material
- Force at gel/bacterium interface=0; back fixed, sides free
- System is infinite in direction out of plane
- Solve with **OOF** (SA Langer, et al.)

Object Oriented Finite Element Analysis for Real Material Microstructures

# Stress Distribution

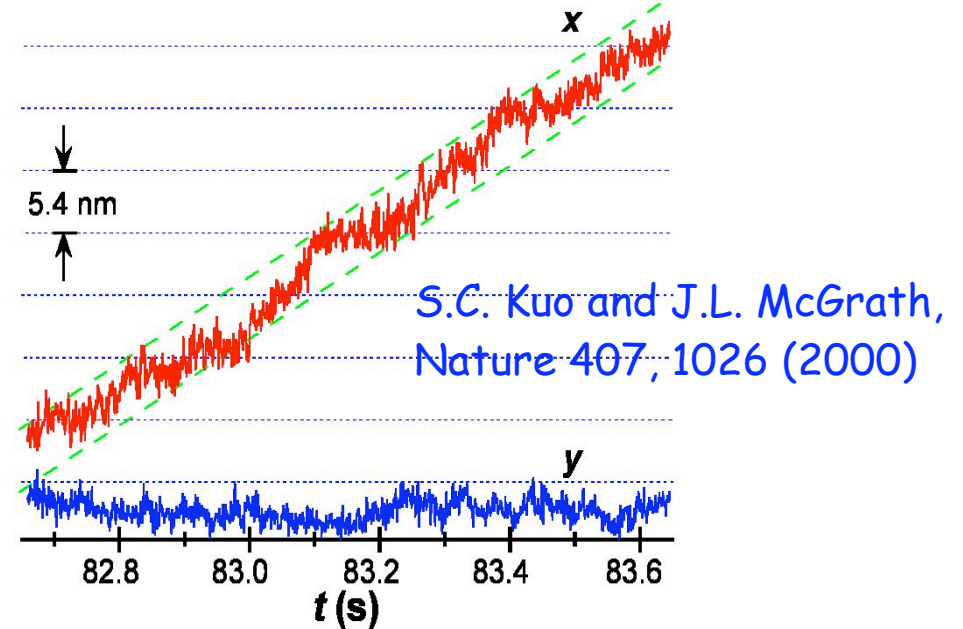
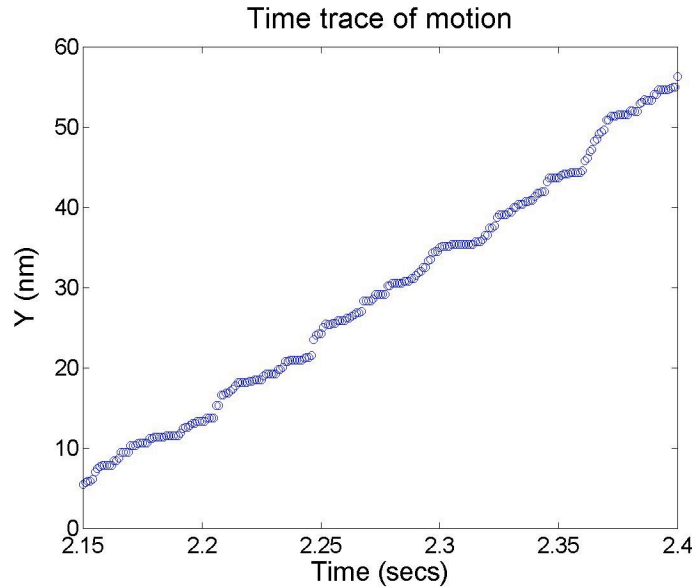
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→ Gel surface under **compressive** stress so force is forwards

← Gel surface under tensile stress so force is backwards

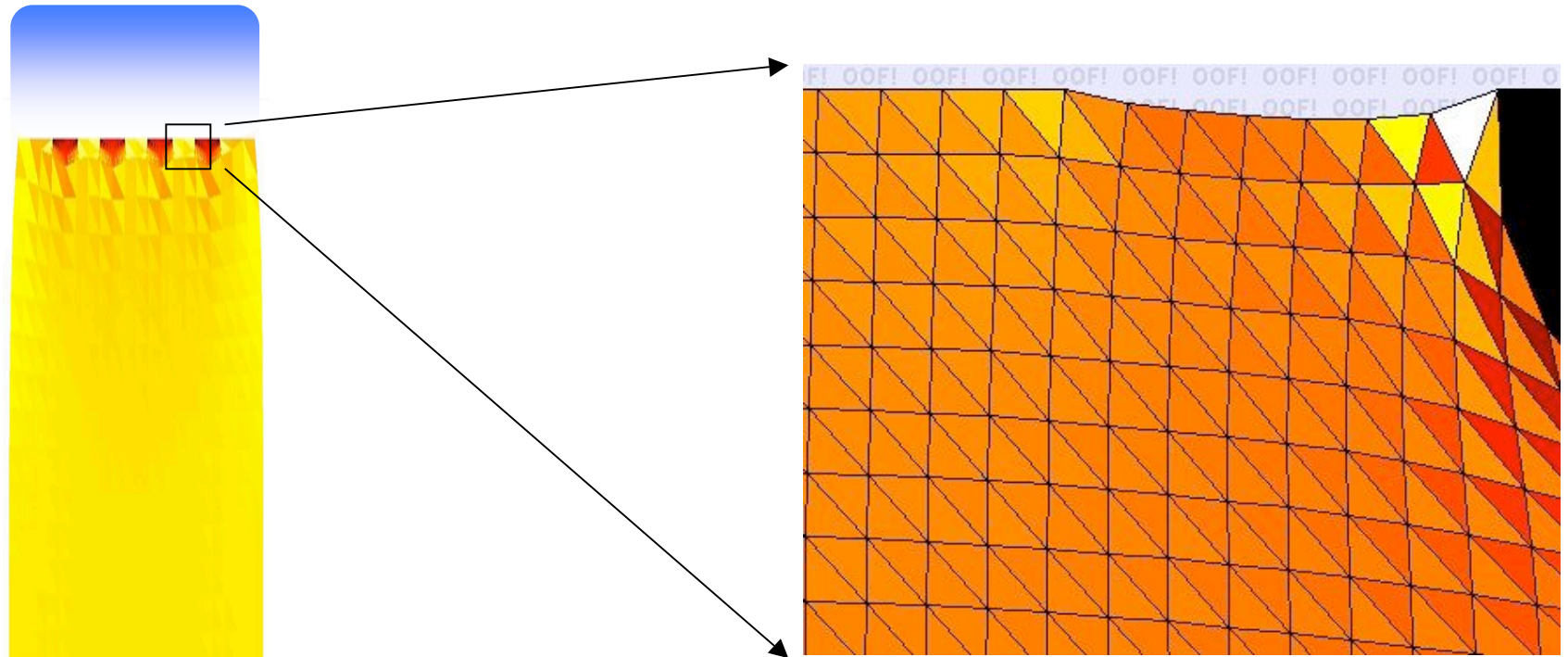
# Nature of Motion



- We see **nanoscale displacement fluctuations** in the motion
  - Size of fluctuation depends on gel modulus, adhesion energy and mesh size
- This has nothing to do with the monomer size since our model does not contain monomers

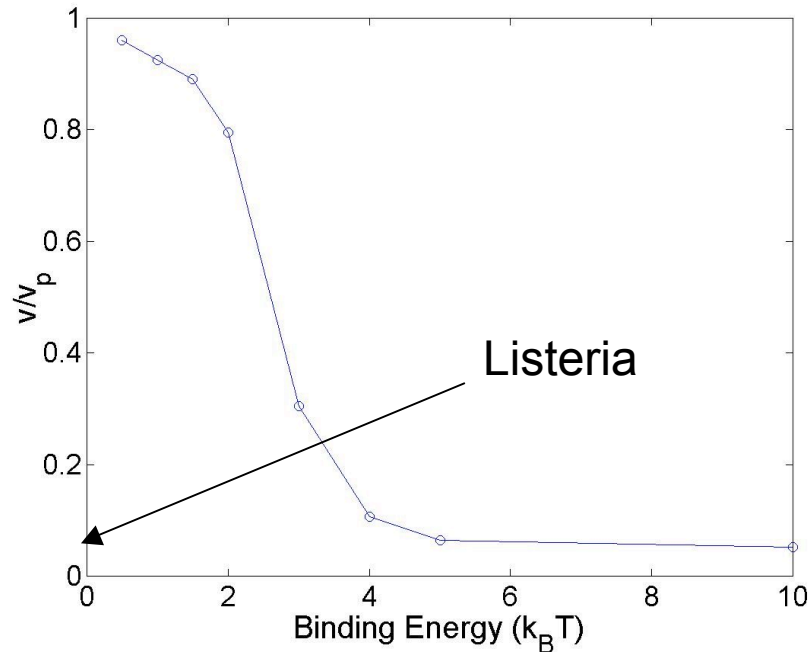
# Why Steps? Adhesive Failure Cascades

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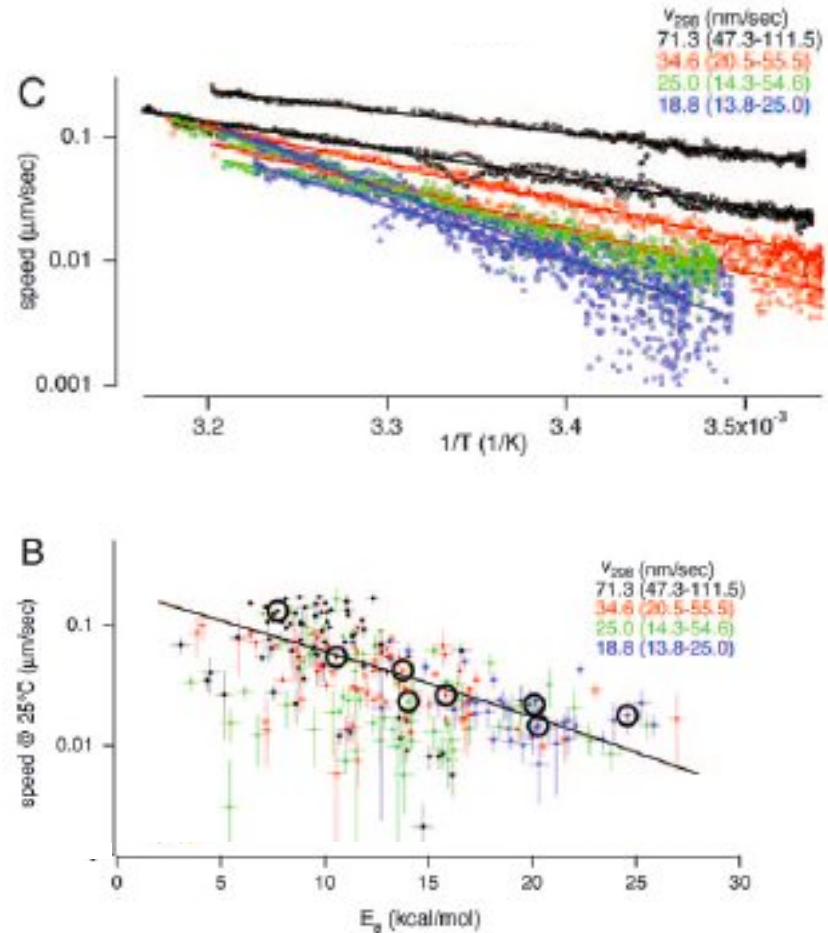




# Speed vs. Binding Energy

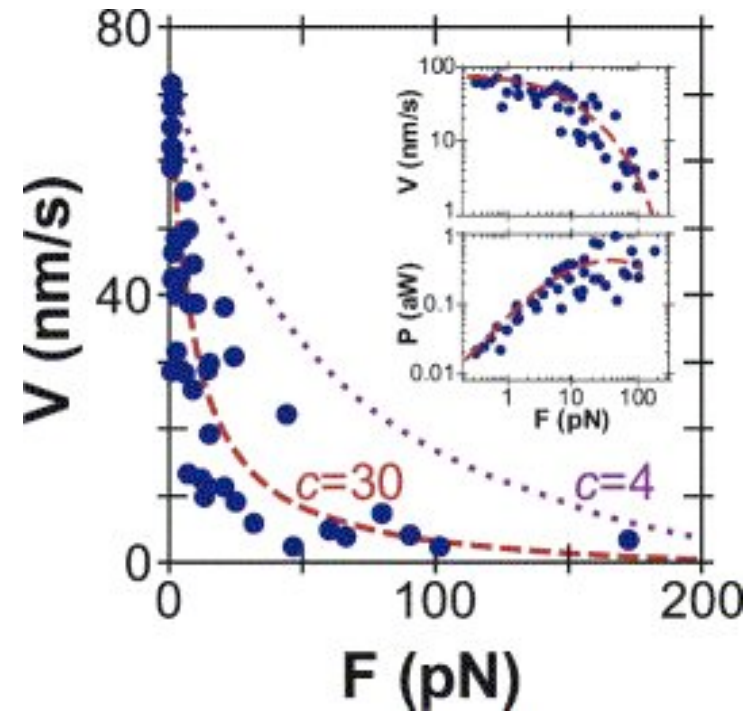
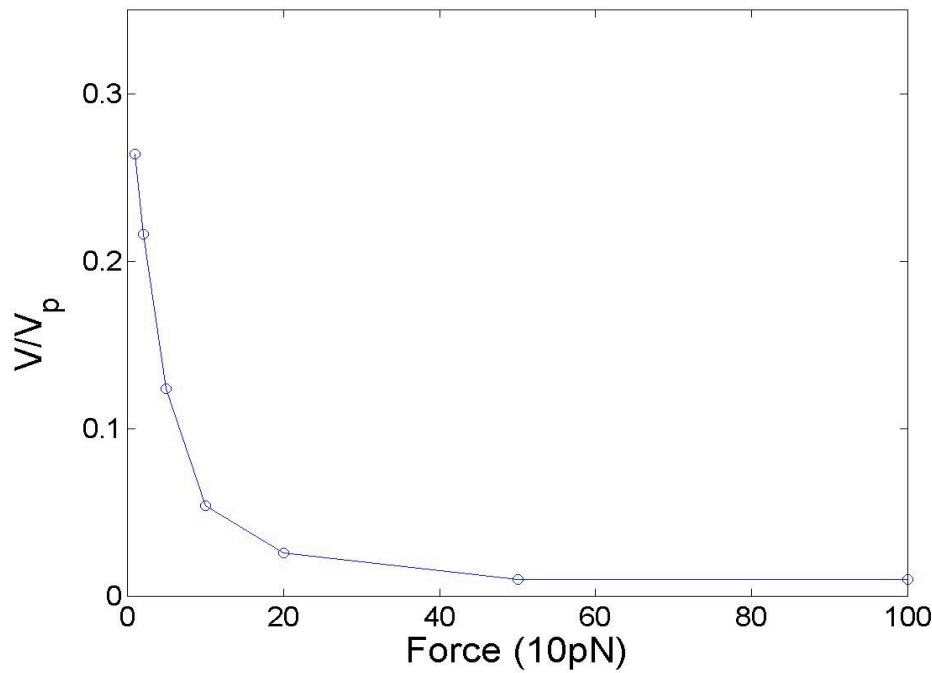


- Speed drops rapidly for small binding energies
- But it is insensitive to large binding energies



F.Soo and J.A. Theriot, PNAS, 102, 45, 16233 (2005)

# Force-Speed Relationship



- Speed decays rapidly at small loads
- But it decays much less rapidly at high load

J. L. McGrath, et al. *Current Biology*, 13, 329-332 (2003).

# Summary (Part I)

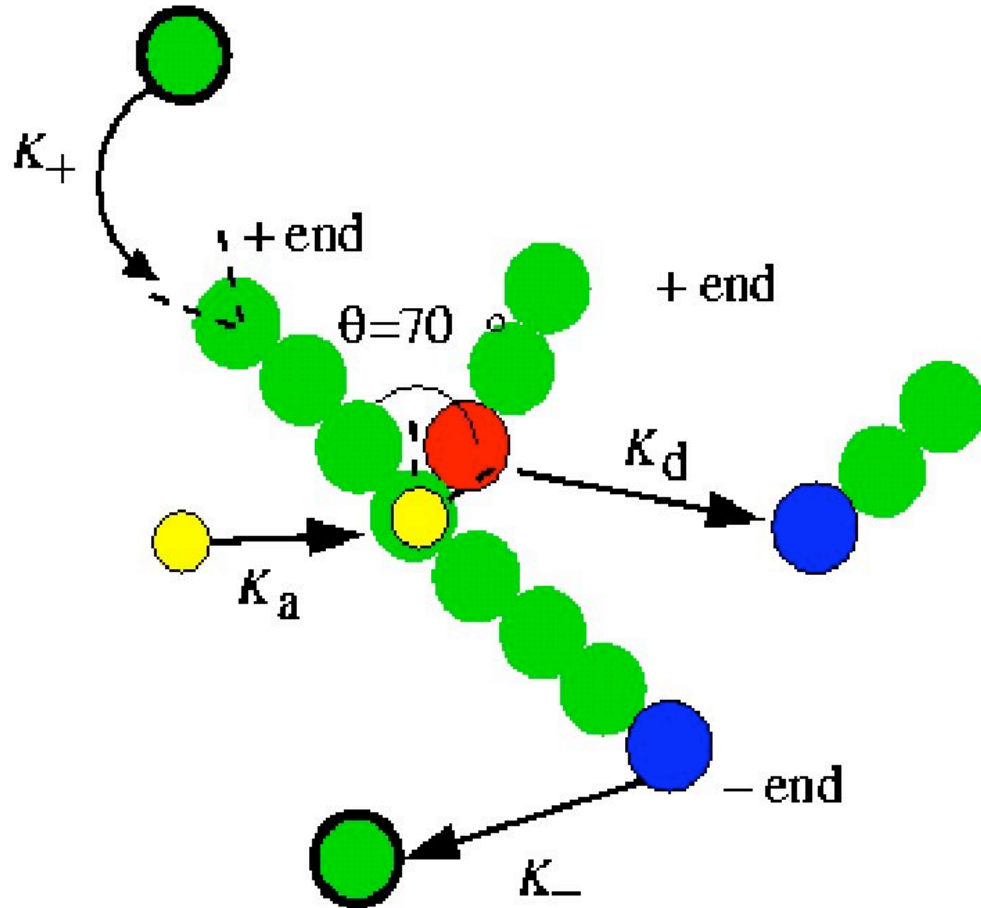
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- Dynamic gel picture appears to capture many features of *Listeria* motility
  - **Nanoscale** displacement fluctuations
  - **Adhesion**-controlled motility
  - **Force-speed** relationship
- **working** and **attached** filaments are **coupled**
- **no special geometry** of bacterium is required for motility

**Ajay Gopinathan(UCSB)**

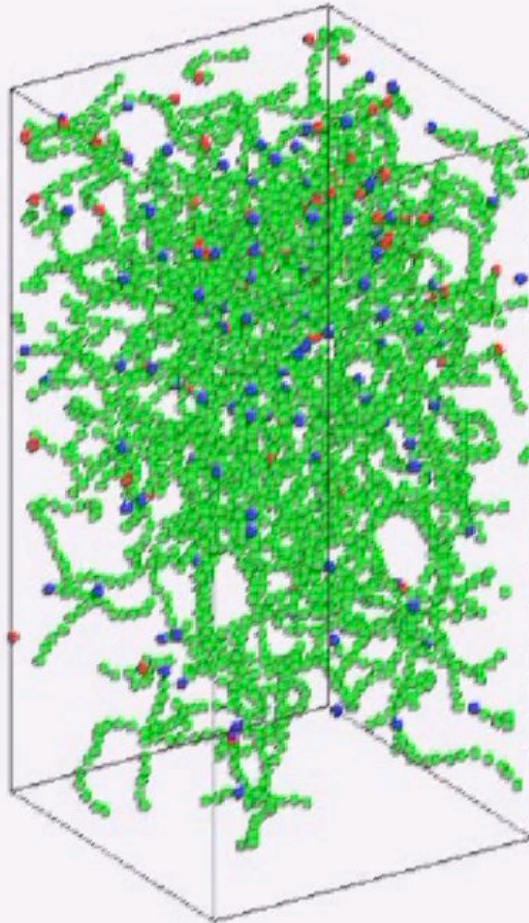
# Brownian Dynamics Simulations (Kun-Chun Lee)

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- Polymerization at + end ( $k_+$ )
- Depolymerization at - end ( $k_-$ )
- Branching ( $k_a$ )
- Debranching ( $k_d$ )
- Capping

# Simulation Setup



Parameter	Experiment	Simulated
$l_p$	1-10 $\mu\text{m}$	0.1-0.3 $\mu\text{m}$
$l_{ave}$	0.1-1 $\mu\text{m}$	0.05-0.1 $\mu\text{m}$
bead size	0.02-1 $\mu\text{m}$	0.3 $\mu\text{m}$
$K_+$	10 $\mu\text{M}^{-1}\text{s}^{-1}$	5 $\mu\text{M}^{-1}\text{s}^{-1}$
$K_-$	1 $\text{s}^{-1}$	100-1000 $\text{s}^{-1}$
[G-Actin]	10 $\mu\text{M}$	600 $\mu\text{M}$
$\frac{K_+[G-Actin]}{K_-}$	100	0.1-1
$K_a$	? $\mu\text{M}^{-1}\text{s}^{-1}$	$\sim K_+$
$K_d$	? $\text{s}^{-1}$	100 $\text{s}^{-1}$
[Arp2/3]	0.1 $\mu\text{M}$	2 $\mu\text{M}^*$
$\frac{K_a[Arp2/3]}{K_d}$	?	0.1-1
$K_{C+}$	3 $\mu\text{M}^{-1}\text{s}^{-1}$	—
$K_{C-}$	0.0004 $\text{s}^{-1}$	0 $\text{s}^{-1}$
[Cap]	0.1 $\mu\text{M}$	—
$K_{C+}[Cap]$	0.3 $\text{s}^{-1}$	10-100 $\text{s}^{-1}$

- Explicit monomers
- Diffusion-controlled polymerization
- Arp2/3 is activated at surface, diffuses and tags filaments

## Comparison with Alberts, et al.

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### Alberts, Odell, PLOS Biol.2, 2055 ('04)

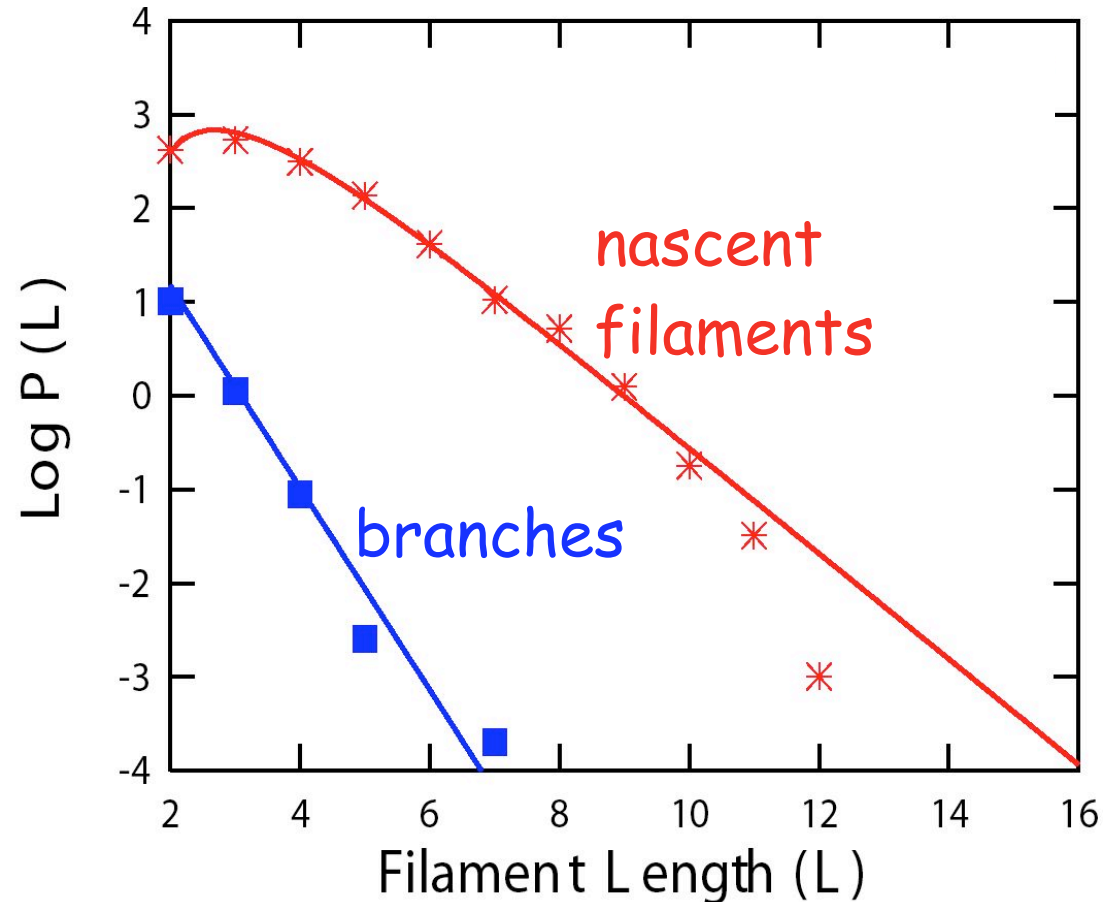
- Realistic rates
- Realistic numbers of filaments
- Concentration fields for arp2/3, G-actin
- Filaments are hard rods
- Forces based on collision resolution rule

### Our work

- Unrealistically high rates
- Small numbers of filaments and system sizes
- Explicit arp2/3, G-actin
- Filaments are semiflexible chains made up of monomers
- Forces determined by potentials

# Benchmarks: Bulk Morphology

- Length distributions



- Branched filament distribution is exponential
- Nascent filament distribution is double-exponential because branches fall off

# Coupled Kinetic Equations

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$$\dot{\rho}_n(L) = k_+ p \rho_m \rho_n(L-1) - k_+ p \rho_m \rho_n(L) + k_- \rho_n(L+1) - k_- \rho_n(L) + k_d \rho_b(L)$$

$$\dot{\rho}_b(L) = k_+ p \rho_m \rho_b(L-1) - k_+ p \rho_m \rho_b(L) + k_- \rho_b(L+1) - k_- \rho_b(L) - k_d \rho_b(L)$$

$$\dot{\rho}_n(2) = -k_+ p \rho_m \rho_n(2) + k_- \rho_n(3) - k_- \rho_n(2) + k_d \rho_b(2)$$

$$\dot{\rho}_b(1) = -k_+ p \rho_m \rho_b(1) - k_d \rho_b(1) + k_a \rho_m \sum_{i=2} i(\rho_n(i) + \rho_b(i))$$

+ conservation of total amount of monomers

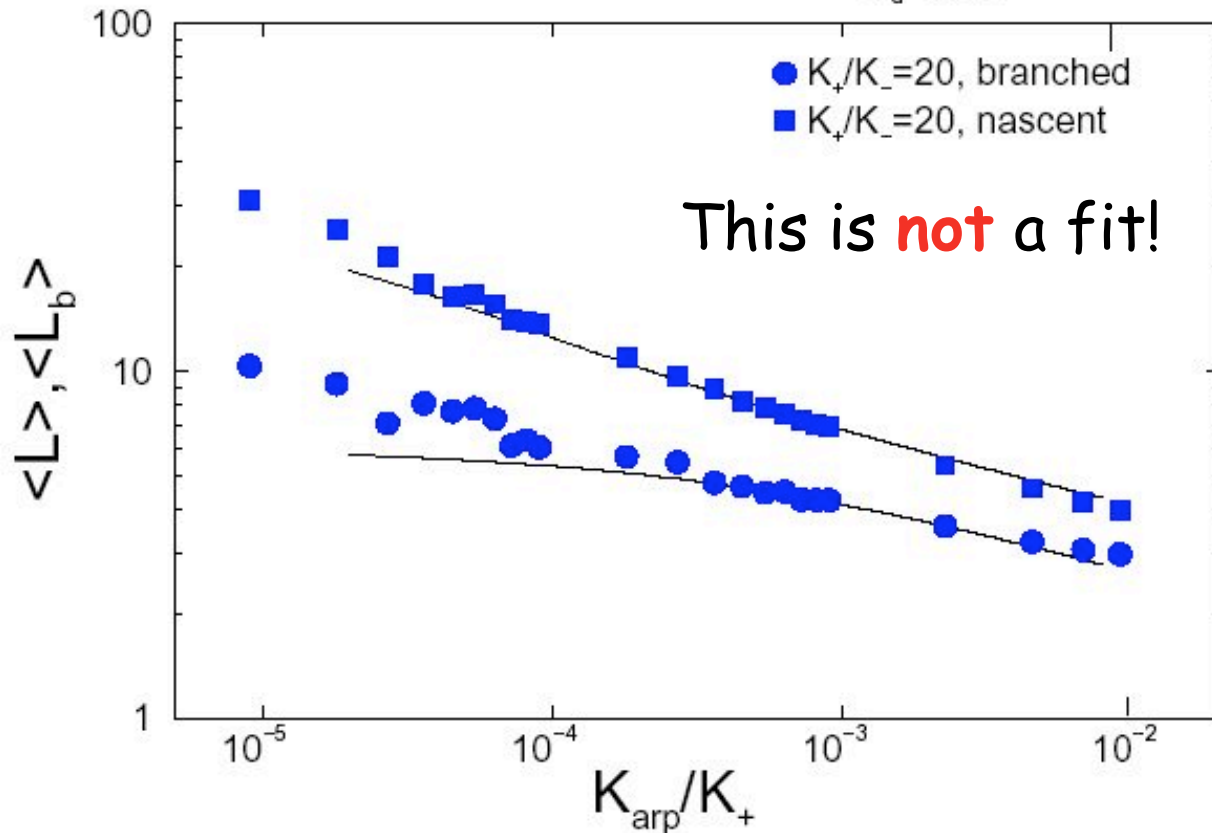
These can be solved

- analytically for steady-state bulk case
- Numerically near a surface moving with steady-state velocity

Ajay Gopinathan, J. M. Schwarz



# Benchmarks: Bulk Morphology II

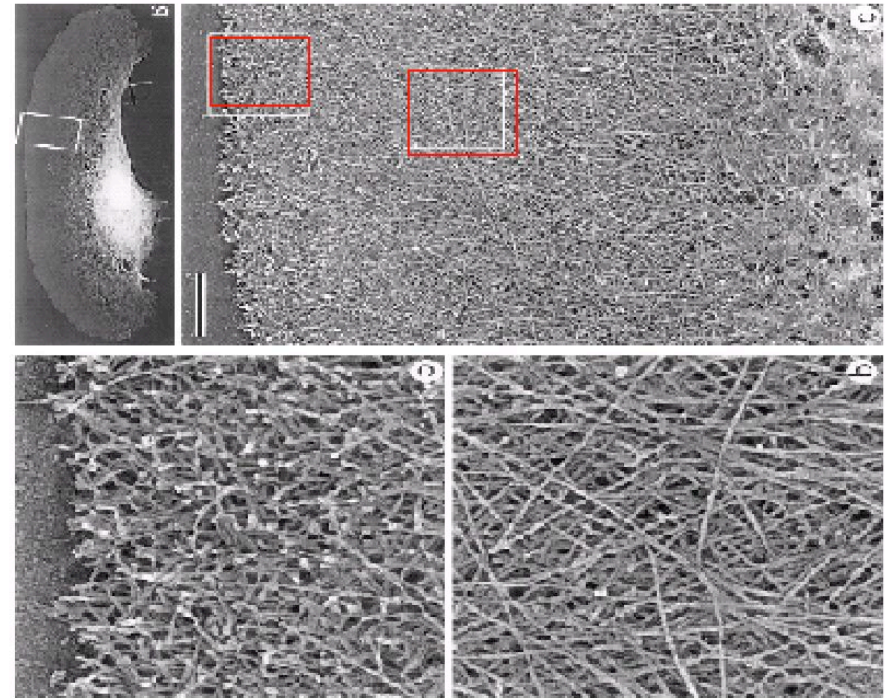
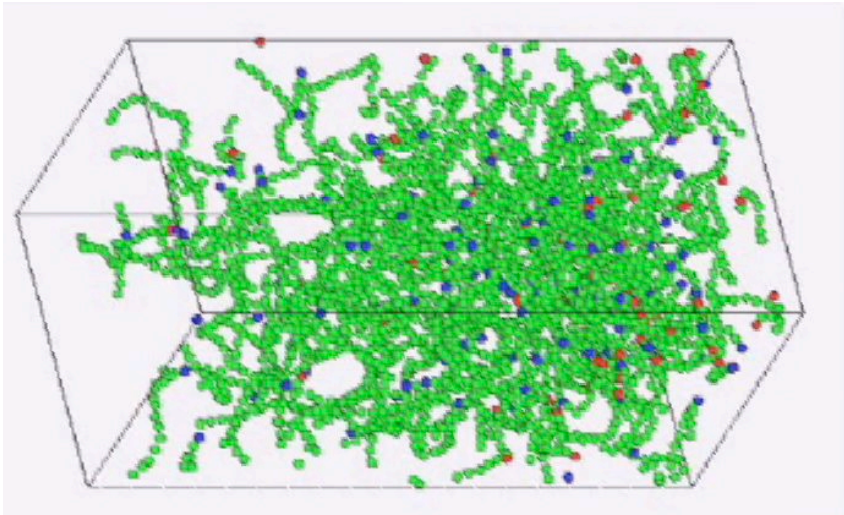


Simulation: points  
Theory: lines

- As Arp2/3 increases, filaments shorten and branch density increases
- Steady-state morphology is robust

# Benchmarks: Moving Surface

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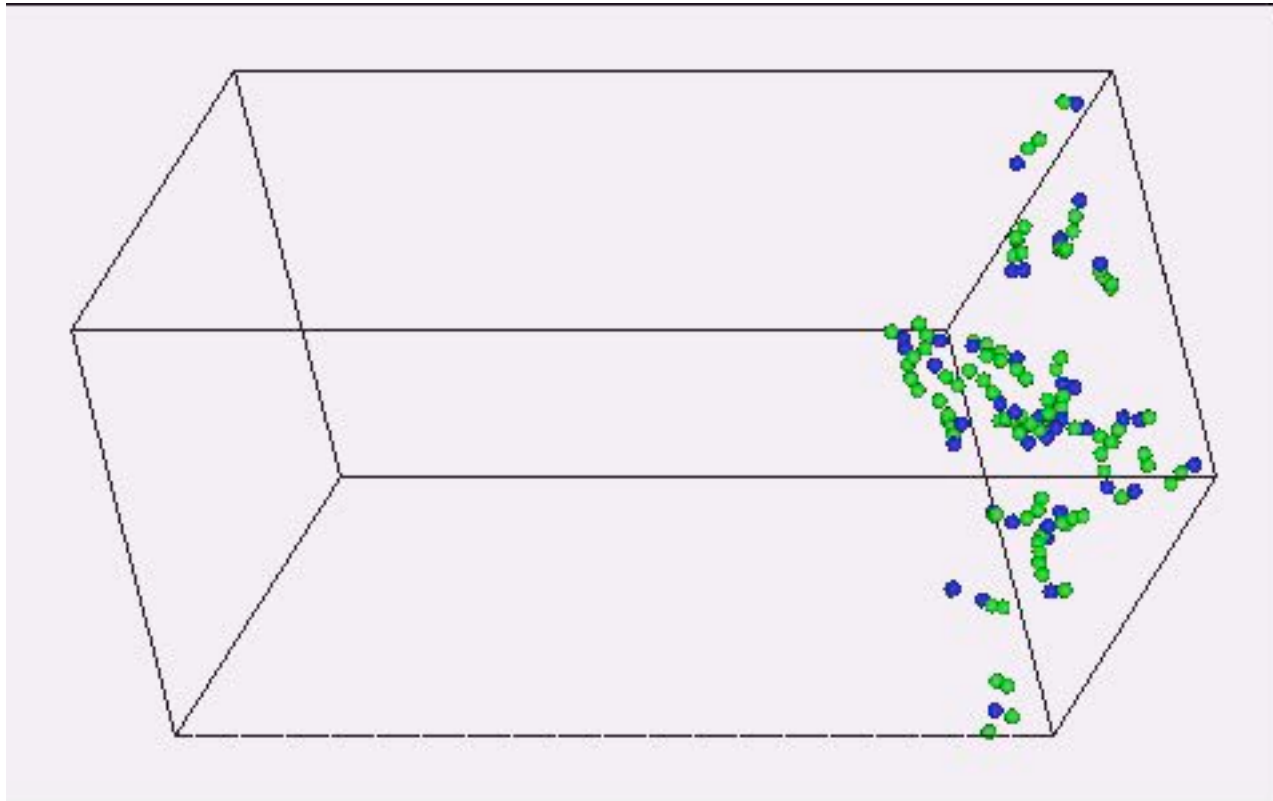


Svitkina et al. J. Cell Biol. 139, 397-405 (1997)

- **Arp2/3** concentrated near surface
- Higher filament density near surface
- Good qualitative agreement with EM images
- Our filaments are more flexible

# Formation of Actin Comet Tail

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Actin monomers

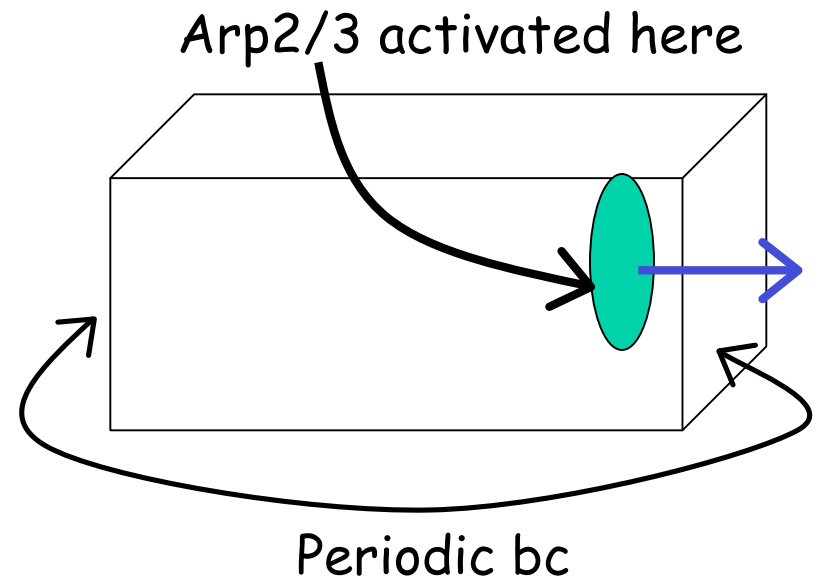
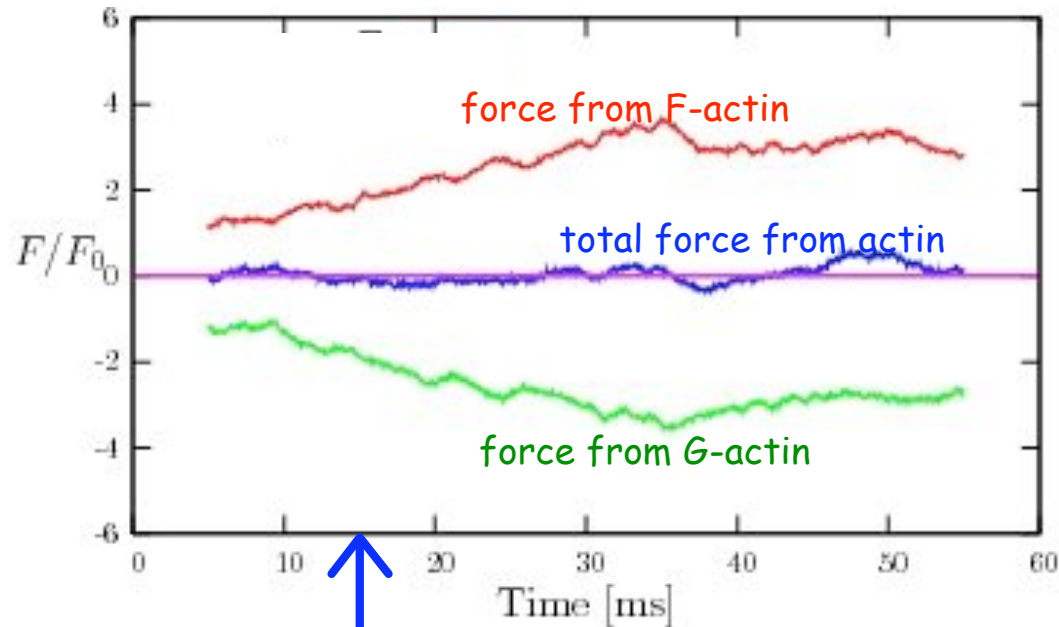
Minus ends

Branch points

Formation of actin comet tail

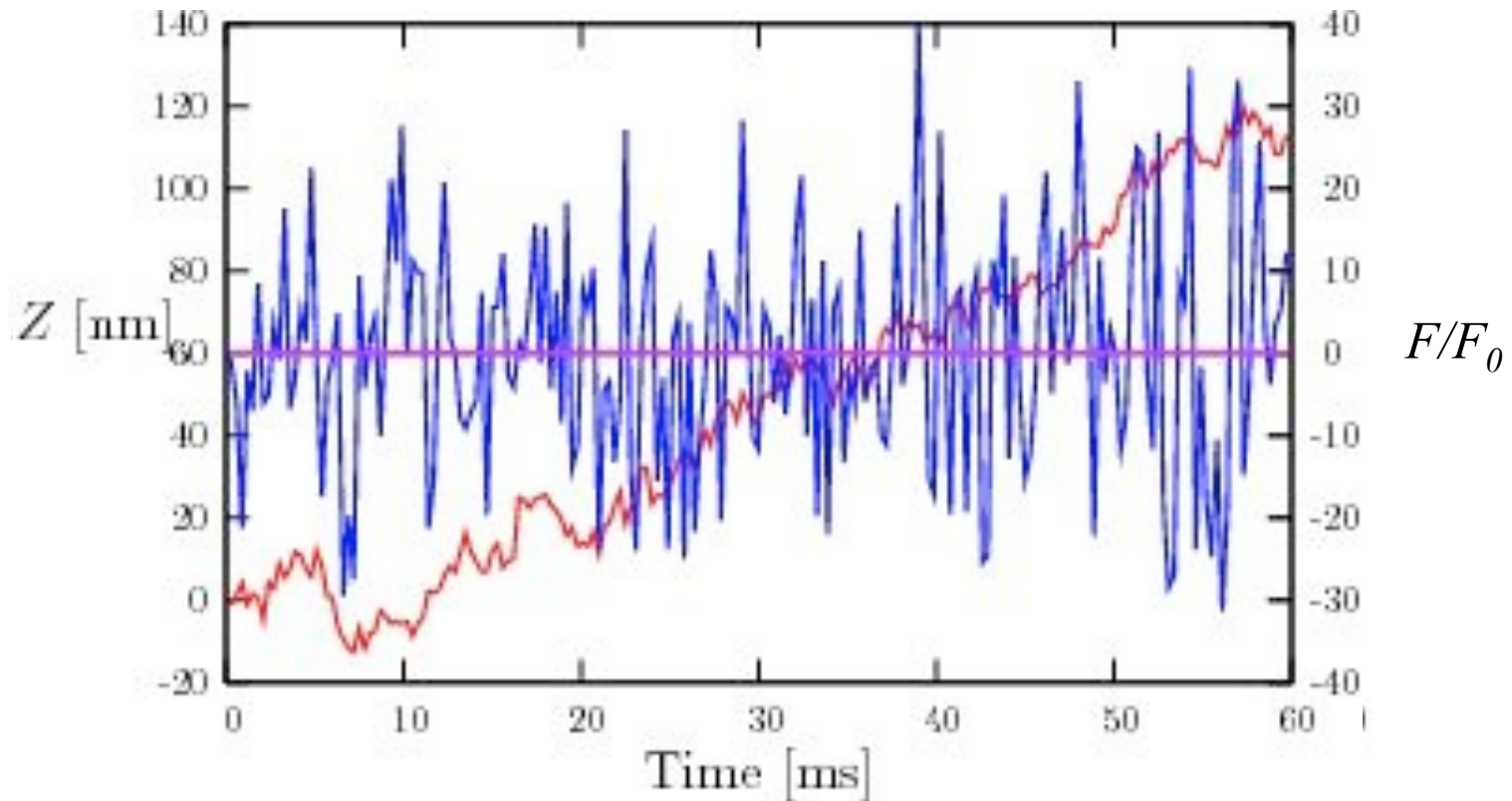
(Free monomers not shown)

# Force vs. Time During Onset of Motion



- Force from **F-actin** is forwards
- Force from **G-actin** is backwards (**G-actin** is depleted behind surface)
- Forces from **F-actin** anticorrelated w/ forces from **G-actin**

# Onset of Motion



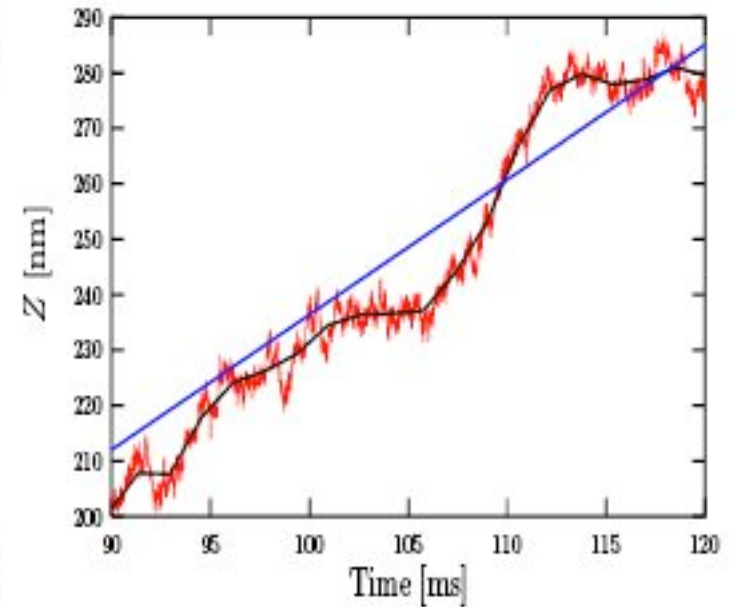
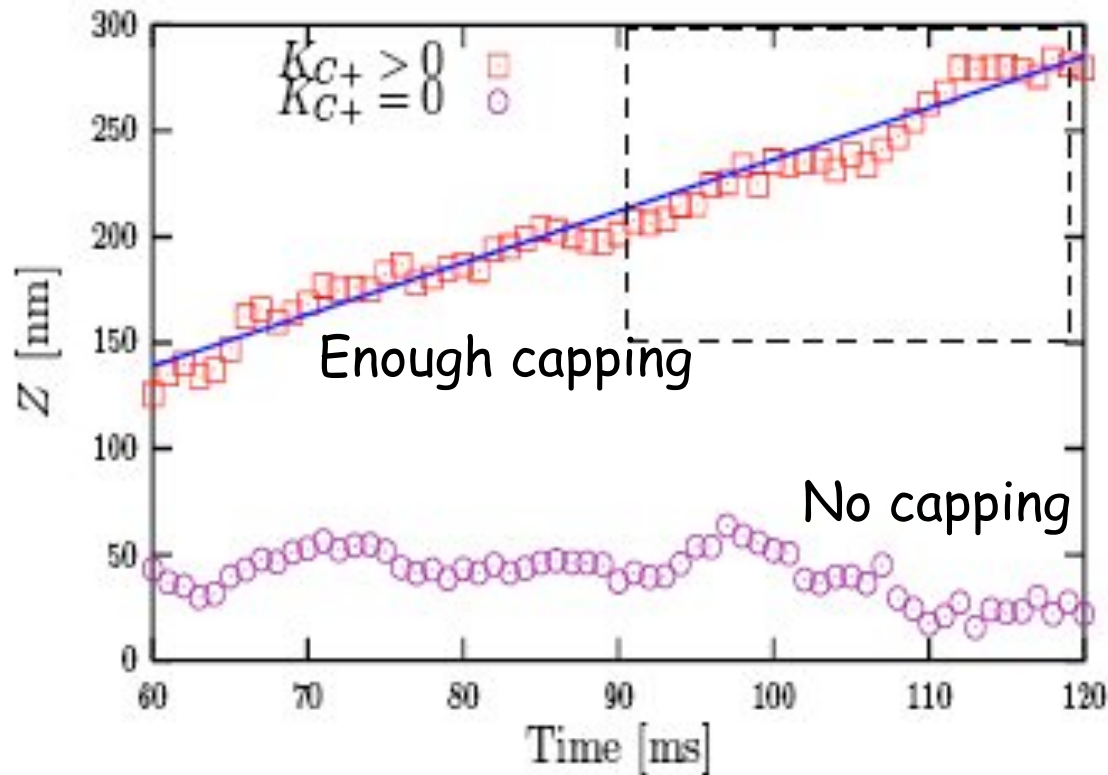
Av. force from filaments  $\langle F_F \rangle / F_0 = 3.12$

Av. force from monomers  $\langle F_G \rangle / F_0 = -2.97$

Total av. force from actin  $\langle F_G + F_F \rangle / F_0 = 0.15 \pm 0.02$

Fluctuations in force are **enormous** compared to average

# Nature of Motion



- Speed is approx  $1\mu\text{m/s}$  (no adhesion)
- Capping is necessary for motion
- Nanoscale displacement fluctuations even without adhesion to surface

# Origin of Displacement Fluctuations

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Dynamical Processes (with capping)

- high [capped plus ends]  $\implies$  more [branches]
- high [branches]  $\implies$  more [plus ends]
- high [plus ends]  $\implies$  more [capped plus ends]



- high [capped plus ends]  $\implies$  more [G-actin] and less [F-actin]
- high [plus ends]  $\implies$  less [G-actin] and more [F-actin]



Fluctuation in [G-actin]/[F-actin] leads to fluctuation in displacement

## Summary (Part II)

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- In steady-state, **G-actin** is **depleted** at surface and is supplied from the back of the tail
- Depletion of **G-actin** near surface leads to backwards force; enhancement of **F-actin** near surface leads to forwards force
- Fluctuations in **[G-actin]/[F-actin]** lead to nm-scale displacement fluctuations even in absence of adhesion

### Open Questions

- Is there an **optimal** F-actin **flexibility** for motility?
- Why is the branching angle **70°**?

**Kun-Chun Lee**

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