Actin Self-Assembly and Listeria Motility

Andrea J. Liu
Department of Physics & Astronomy
University of Pennsylvania

Ajay Gopinathan — Physics & MRL, UCSB
Kun-Chun Lee — Physics & Astronomy, UPenn
J. M. Schwarz — Physics, Syracuse University

Itamar Borukhov — Compugen, Inc.
Robijn F. Bruinsma — Physics, UCLA
William M. Gelbart — Chemistry & Biochemistry, UCLA

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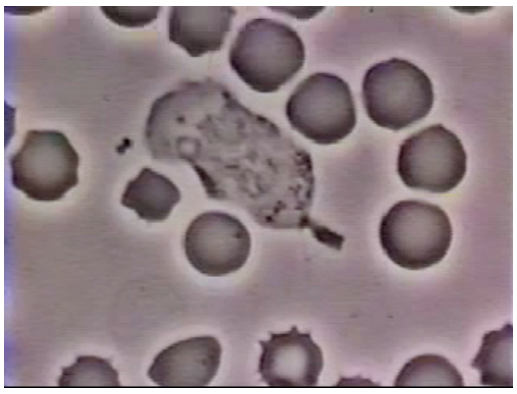
Cell Crawling

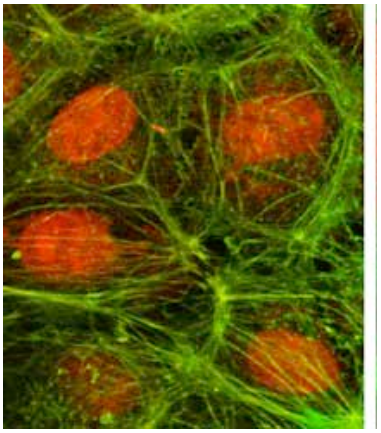


Neutrophil chasing *Staphylococcus aurea*

David Rogers, Vanderbilt University, 1959

http://www.chem.uic.edu/fenteany/research/cell_migration/neutrophil.html

Cytoskeleton



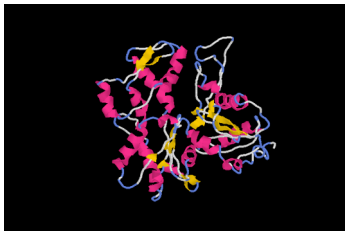
Courtesy of M. Gimbone

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

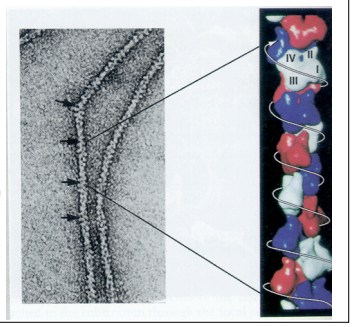
¿ How is polymerization converted into motion?

Physical Properties of F-Actin

G-actin
55Åx55Åx35Å
12-13 negative charges

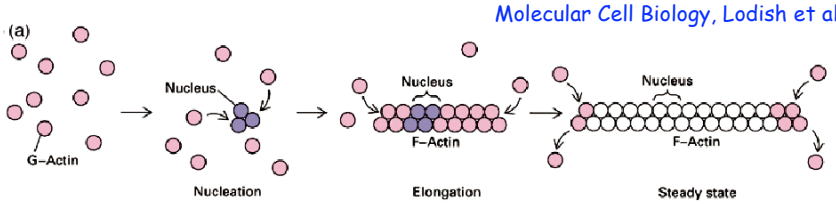


- F-actin is double-helix of G-actin
- Diameter is D=8 nm
- Length is L=100 nm to μm
- Persistence length is 2-18 μm (rigid)
- Charge density is - 1 e/2.5 Å



Actin Polymerization and Depolymerization

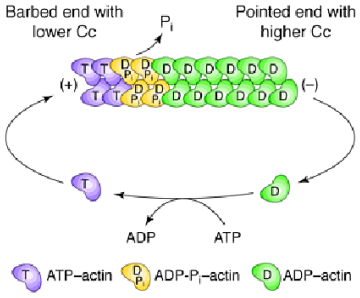
Molecular Cell Biology, Lodish et al



- Constant turnover of actin monomers
- Filaments created and dismantled
- A 3μm long filament turns over in 1 min *in vivo*

From "Understanding How Life Works," M. Hoagland and B. Dodson

Treadmilling

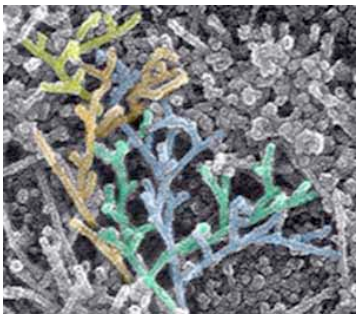


Actin filaments tend to polymerize at one end (barbed end) and depolymerize at the other (pointed end)

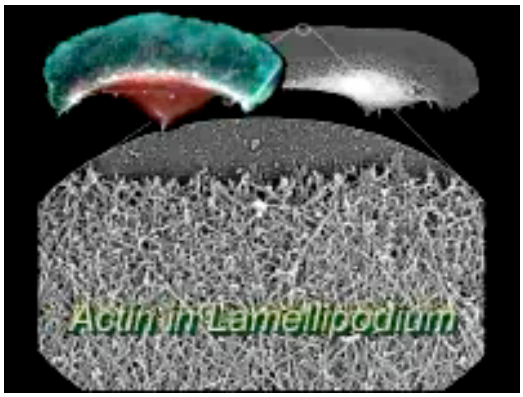
From Chen, Bernstein and Bamberg, *Trends Biochem Sci* 2000 Jan., volume 25, pages 19-23.

- ATP hydrolysis provides polarity to filament growth
- Growing ends localized near cell membrane

How are Growing Ends Localized at Leading Edge?



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



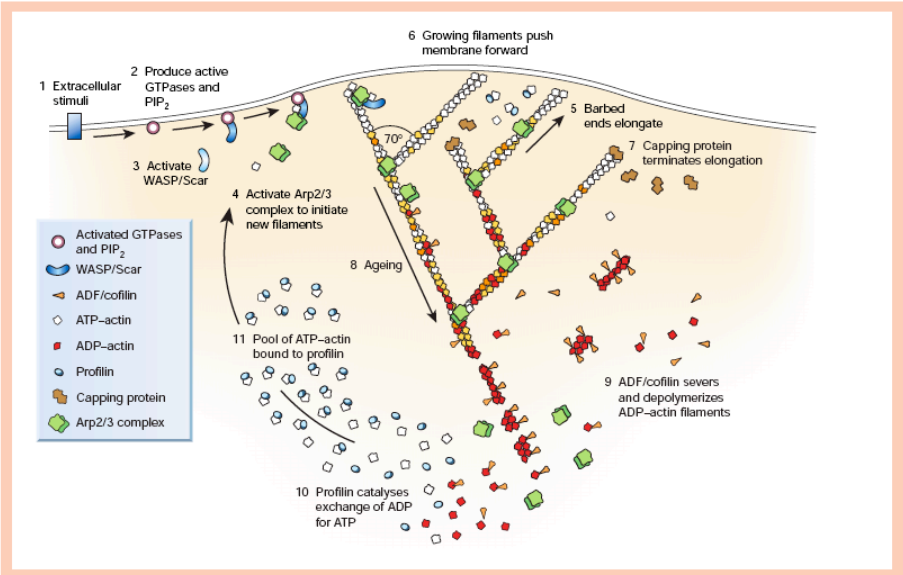
Courtesy: T. M. Svitkina, G.G. Borisy

www.borisylab.northwestern.edu

Xenopus keratocytes

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

Dendritic Nucleation Model: Participating Proteins



T. D. Pollard, L. Blanchoin, R. D. Mullins, Ann. Rev. Biophys. Biomol. Struct. **29**, 545-576 (2000)

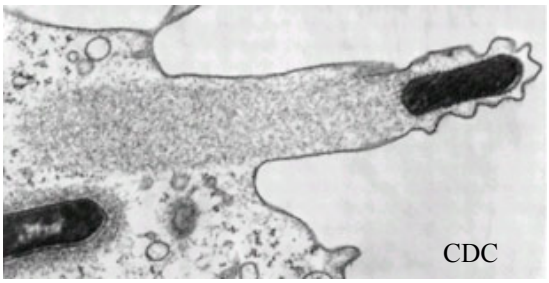
Dendritic Nucleation Model

- Arp2/3 complex nucleates new plus ends
- Capping protein kills off older plus ends
- Severing proteins help break up older filaments by creating 2 minus ends in place of 1
- Profilin turns ADP-G-actin into ATP-G-actin

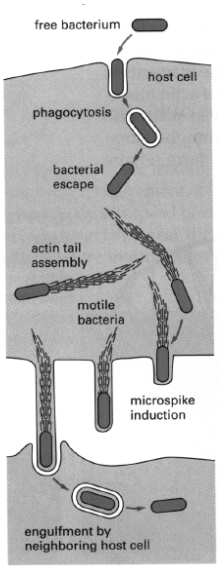
- Subtleties:
  - Arp2/3 binds more strongly to ATP-actin
  - Severing proteins bind more strongly to ADP-actin

What is the evidence for this picture?

Actin and Listeria Motility



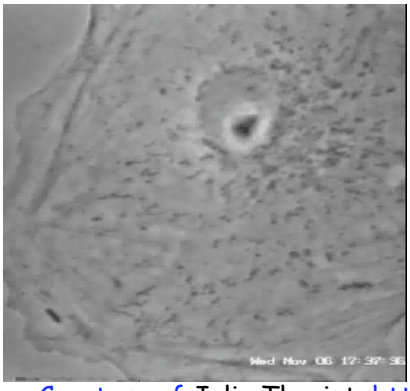
Life cycle of
Listeria monocytogenes



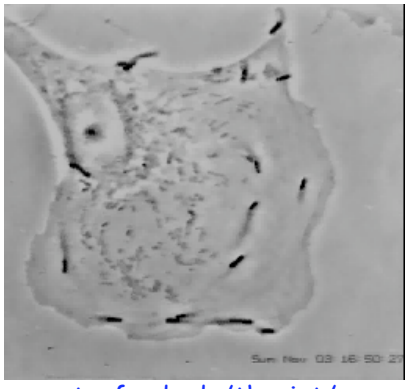
Uses actin polymerization to move!
Same physics as cell crawling

How Listeria Spreads from Cell to Cell

x900



x150

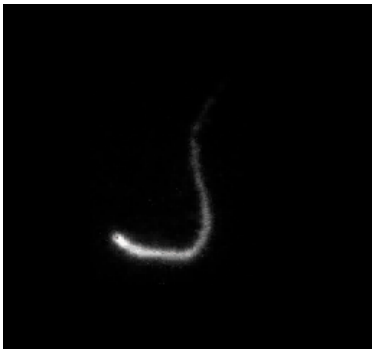


Courtesy of Julie Theriot, http://cmgm.stanford.edu/theriot/

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

Comet tail is nearly **stationary**

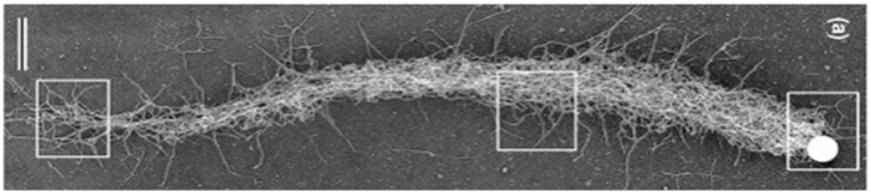
Polystyrene Beads Can Do It, Too!



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

x60

Actin comet tail has branchy structure similar to that of 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

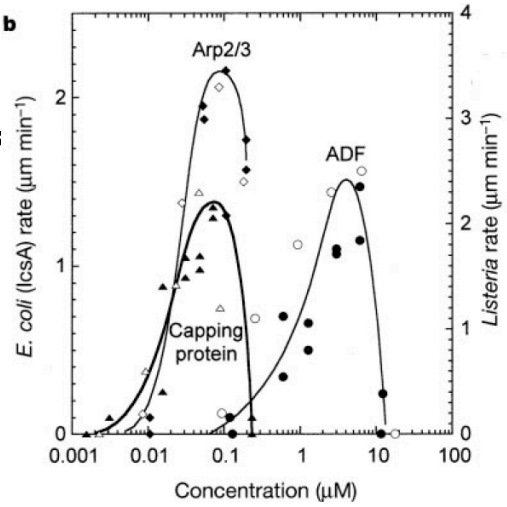
-Arp2/3 makes new growing ends

-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



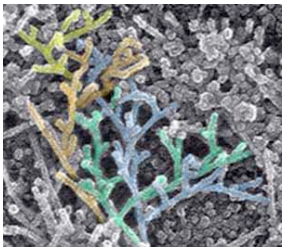
Loisel, Boujemaa, Pantaloni, Carlier, Nature, **401**, 613 (1999).

Questions

- What is the morphology of the branched actin network?

  Ajay Gopinathan,

  Kun-Chun Lee



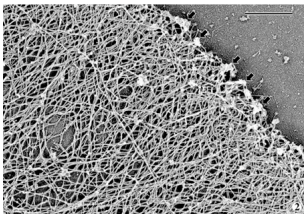
T. M. Svitkina, G. G. Borisy, J. Cell Biol. 145, 1009 (1999).

- How is morphology coupled to motility?

  J. M. Schwarz,

  Ajay Gopinathan

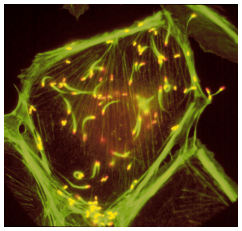
  Kun-Chun Lee



T. M. Svitkina, A. B. Verkhovsky, G. G. Borisy, J. Struct. Biol. 115, 290 (1995).

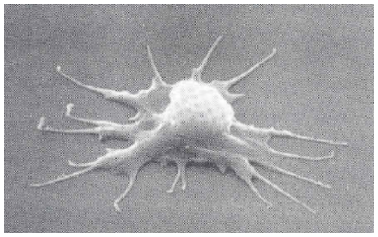
Questions

- How is polymerization converted to motion?
  - Kun-Chun Lee
  - Ajay Gopinathan



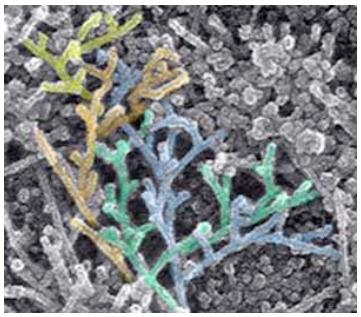
J. M. Theriot

- Filapodia vs. Lamellipodia?
  - Itamar Borukhov
  - Robijn F. Bruinsma
  - William M. Gelbart



Actin Morphology

Ajay Gopinathan, Kun-Chun Lee, Jen Schwarz



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

What is dependence of
- filament length
- distance between branches
- branch length

on concentrations of
- Actin
- Arp2/3
- Capping protein
- Severing protein

What is the morphology of the branched actin network and how is it coupled to motility?

Coupled Kinetic Equations (mean-field)

$$\dot{\rho_u}(L) = -k_+\rho_m(\rho_u(L) - \rho_u(L-1)) + k_-(\rho_u(L+1) - \rho_u(L)) + k_d\rho_b(L) - k_s\sum_{L'=1}^{L-1} p(L')\rho_u(L)$$
$$+ \sum_{L'=L+2}^{\infty} k_s p(L)\rho_b(L') + \sum_{L'=L+1}^{\infty} k_s(p(L)+p(L'-L))\rho_u(L')$$

$$\dot{\rho_b}(L) = -k_+\rho_m(\rho_b(L) - \rho_b(L-1)) - k_d\rho_b(L) - k_s\sum_{L'=1}^{L-2} p(L')\rho_b(L) + \sum_{L'=L+1}^{\infty} k_s p(L'-L)\rho_b(L')$$

$$\dot{\rho_u}(2) = -k_+\rho_m(\rho_u(2)) + k_-\rho_u(3) - k_{diss}\rho_u(2) + k_d\rho_b(2) + k_n\rho_m^2$$
$$+ \sum_{L'=4}^{\infty} k_s p(2)\rho_b(L') + \sum_{L'=3}^{\infty} k_s(p(2)+p(L'-2))\rho_u(L')$$

$$\dot{\rho_b}(2) = -k_+\rho_m(\rho_b(2)) - k_d\rho_b(2) + k_{arp}\rho_m^2\sum_{L=2}^{\infty}(\sum_{L'=1}^{L} 1 - p(L'))(\rho_u(L)+\rho_b(L)) + \sum_{L'=3}^{\infty} k_s p(L'-2)\rho_b(L')$$

+ eqn to fix total amount of monomers

$p(L) = 1 - e^{-L/\ell_c}$ is prob. monomer distance L from + end is ADP-actin

$\ell_c = k_+\rho_m / k_{pr}$

Other Approaches

These do not include 1 or more of the following processes:
branching, severing, capping

A. Mogilner, L. Edelstein-Keshet, Biophys. J. **83**, 1237 (2002).

L. Edelstein-Keshet, G. B. Ermentrout, J. Math. Biol. **43**, 325 (2001).

A. E. Carlsson, PRL **92**, 238102 (2004).

A. E. Carlsson, Biophys. J. **89**, 130 (2005).

A. E. Carlsson, M. A. Wear, J. A. Cooper, Biophys. J. **86**, 1074 (2004).

This describes morphology only in terms of total amount of actin in polymerized form

A. E. Carlsson, Biophys. J. **90**, 413 (2006).

Analysis: Coupled Kinetic Equations

- In absence of severing or nucleotide dependence, equations can be solved analytically

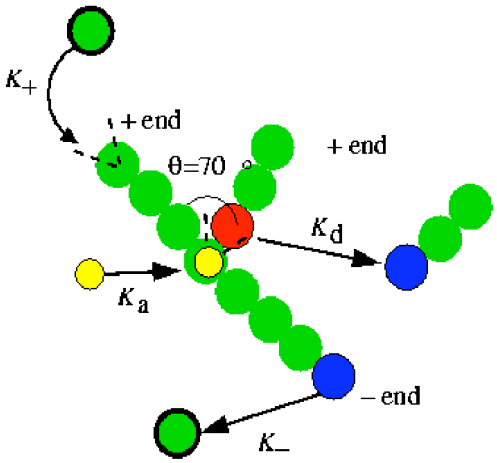
$$\rho_u(L) = Ae^{-L/\lambda_u} + Be^{-L/\lambda_b}$$

$$\rho_b(L) = Ce^{-L/\lambda_b}$$

$$\lambda_u = \frac{1}{\log \frac{k_-}{k_+ \rho_m^*}} \approx \frac{k_+ \rho_m^*}{k_- - k_+ \rho_m^*} \qquad \lambda_b = \frac{1}{\log \frac{k_d + k_+ \rho_m^*}{k_+ \rho_m^*}} \cong \frac{k_+ \rho_m^*}{k_d}$$

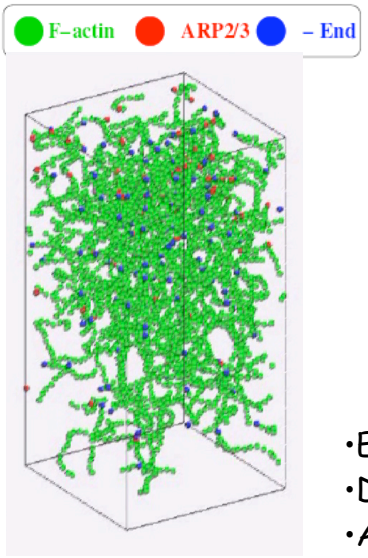
- Similar to solutions for living polymers
  - e.g. R. L. Scott, J. Phys. Chem. 69, 261 (1965)
    M. E. Cates, S. J. Candau, J. Phys. Cond. Mat. 2, 6869 (1990)

Brownian Dynamics Simulations (Kun-Chun Lee)



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

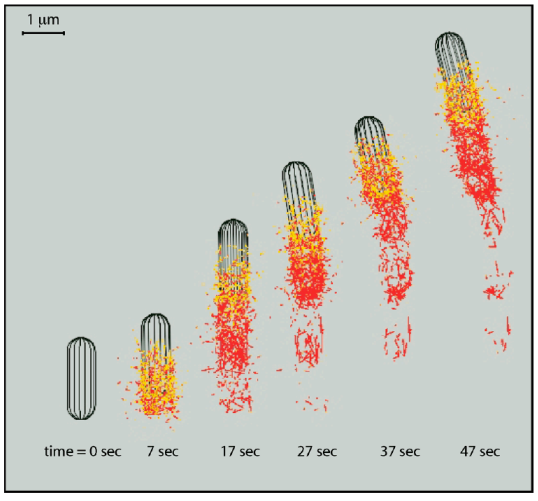
Simulation Setup



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

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

Other Approaches



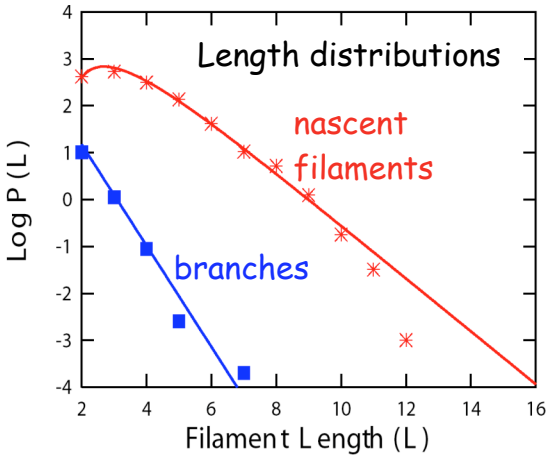
J. B. Alberts, G. M. Odell, PLOS Biology, **2**, 2054 (2004).

Comparison with Alberts, et al.

Alberts & Odell	Our work
• Realistic rates	• Unrealistically high rates
• Realistic numbers of filaments	• Small numbers of filaments and system sizes
• Concentration fields for arp2/3, G-actin	• Explicit arp2/3, G-actin
• Filaments are hard rods	• Filaments are semiflexible chains made up of monomers
• Forces based on collision resolution rule	• Forces determined by potentials

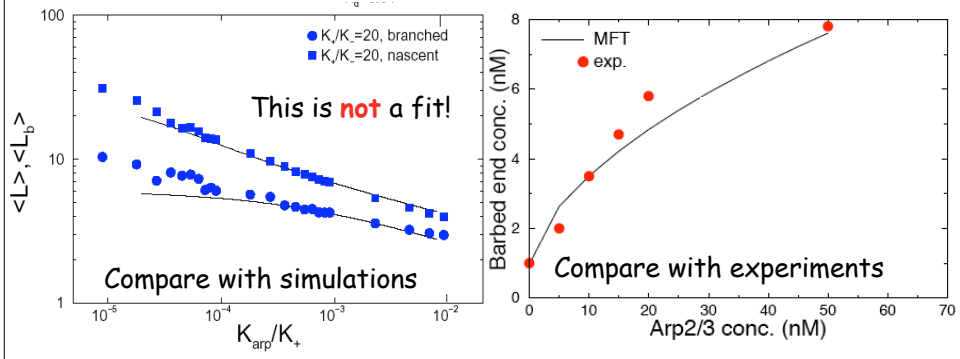
Bulk Filament Length Distributions

- Branched filament distribution is exponential
- Free-end filament distribution is double-exponential because branches fall off



Brownian dynamics simulations (points) agree well with mean-field solution (lines) with no adjustable parameters

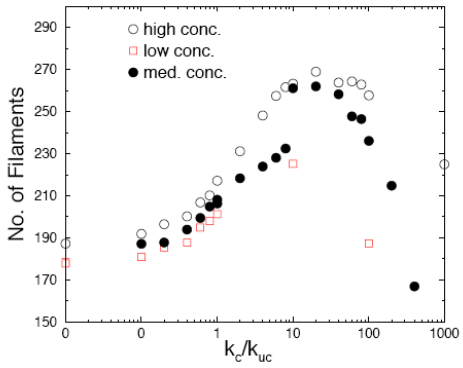
Bulk Morphology: Dependence on Arp2/3



L. Blanchoin,K. J. Amann, H. N. Higgs, J.-B. Marchand, D. A. Kaiser, T. D. Pollard, Nature, **404**, 1007 (2000).

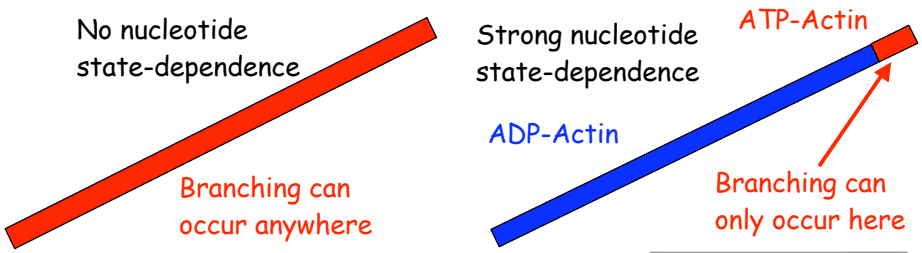
- As Arp2/3 increases, filaments shorten and branch density increases
- Steady-state morphology is robust

Capping Dependence



- At low capping rates, old filaments keep growing
- At high capping rates, nothing can grow
- At intermediate capping rates, most of growth goes into new or young branches

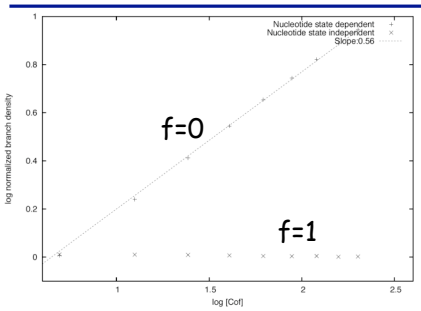
Severing and Nucleotide-state dependence

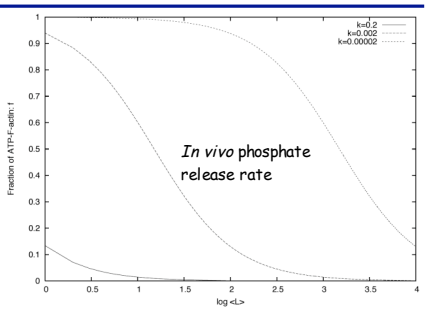


- Severing and branching coupled by nucleotide state dep.
  - If no nucleotide-state dep., branch density depends on density of actin filaments
  - If nucleotide-state dep., branch density depends on number of actin filaments

L. Blanchoin, K. J. Amann, H. N. Higgs, J.-B. Marchand, D. A. Kaiser, T. D. Pollard, Nature **404**, 1007 (2000).

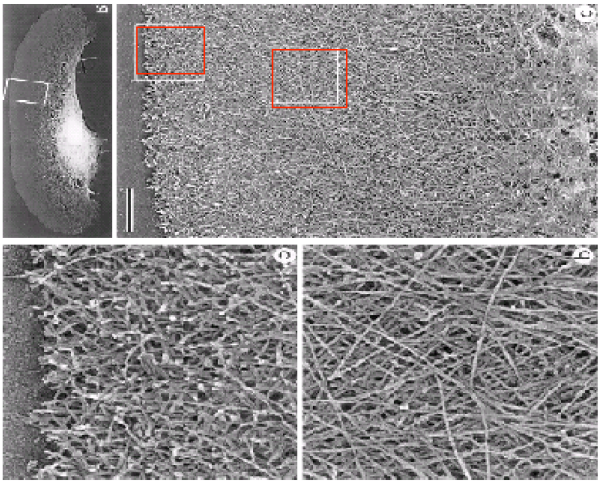
Severing and Nucleotide-state dependence





- Severing and branching coupled by nucleotide state dep.
  - Branch density is sensitive to severing conc. $\approx \sqrt{k_s k_{\mathrm{arp}}}$
  - System switches from side-branching (f=1) to end-branching regime (f=0) as average length increases
  - *In vivo* phosphate release rate and filament lengths optimize branching/severing coupling; can control morphology with severing protein concentration

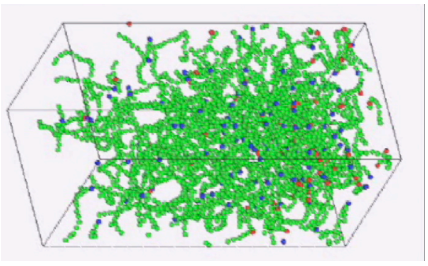
Morphology Near Moving Surface

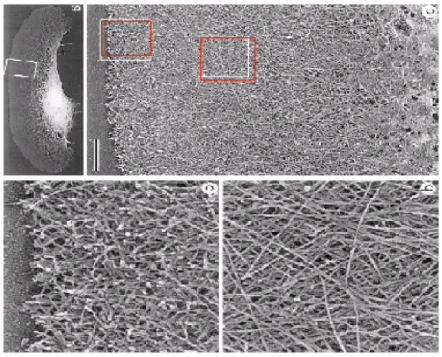


Svitkina et al. J. Cell Biol. 139, 397-405 (1997)

- Denser, shorter branches near surface
- Sparser, longer branches far away
- Lamellipodium thickness $\approx 10\mu m$

Simulation Results for Surface Morphology





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

Mean-Field Analysis of Surface Morphology

- Bulk equations can be generalized to depend on distance z from surface, e.g.

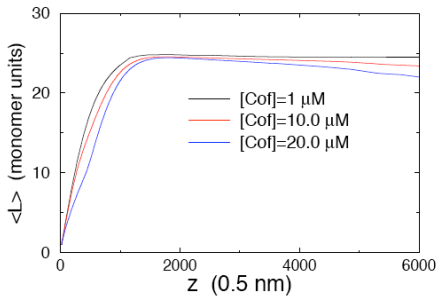
$$\rho(L,z,t) = \rho(L,z-v\,dt,t-dt) + k_1\rho_m(z)\Big(\rho(L-dL,z-v\,dt+dL\cos(\theta),t-dt) - \rho(L,z-v\,dt,t-dt)\Big) + k_{-1}\Big(\rho(L+dL,z-v\,dt,t-dt) - \rho(L,z-v\,dt,t-dt)\Big) + k_d\rho_b(L,z-v\,dt,t-dt),$$

- In steady-state, surface speed and morphology must be solved self-consistently
  - If speed is too slow, profile builds up with time
  - If speed is too fast, profile gets left behind

Monomer Transport to Leading Edge

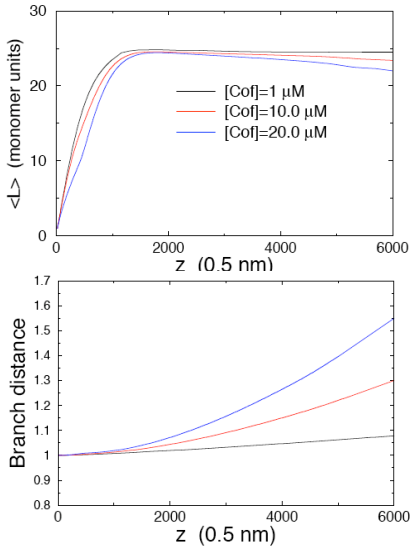
D. Zicha, I. M. Dobbie, M. R. Holt, J. Monypenny, D. Y. H. Soong, C. Gray, G. A. Dunn, Science **300**, 142 (2003).

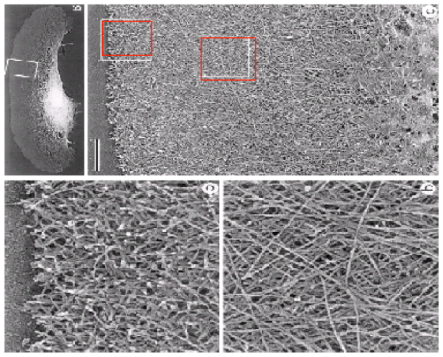
Width of Lamellipodium



Width approx 10 μm

Morphology in Lamellipodium





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

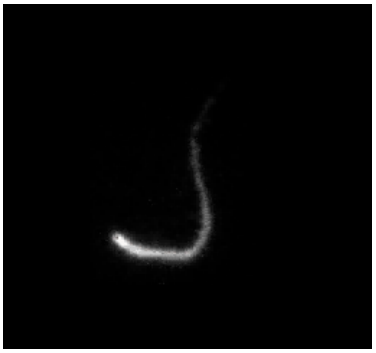
- Filaments lengthen away from surface
- Distance between branches increases away from surface

Summary (Part I)

- Mean-field description of dendritic-nucleation model works well, both in bulk and at surface
- For rapid ATP-ADP conversion, branching occurs near growing tips
- Severing is efficient way of controlling number of filaments & filament length -> amount of branching
- Steady-state surface morphology & speed are coupled
- Monomer current speeds of 1μm/sec required for realistic surface morphology and speeds
- Severing is necessary to achieve realistic morphologies

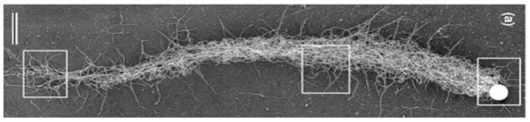
Ajay Gopinathan, Kun-Chun Lee, J. M. Schwarz

Motility



Courtesy of Julie Theriot, x60
http://cmgm.stanford.edu/theriot/

Kun-Chun Lee, Ajay Gopinathan



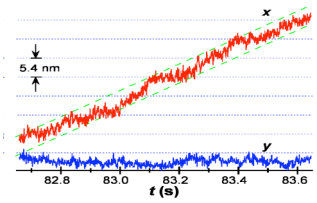
L. A. Cameron, T. M. Svitkina, D. Vignjevic, J. A. Theriot, G. G. Borisy, Current Biology, **11**, 130 (2001).

How does polymerization into branched structures lead to force generation and motility?

Signatures of the motility

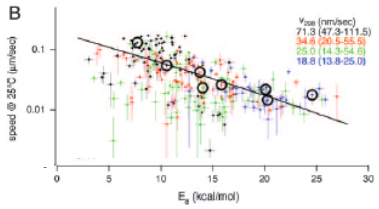
- Nanoscale displacement fluctuations

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



- Adhesion controls motility

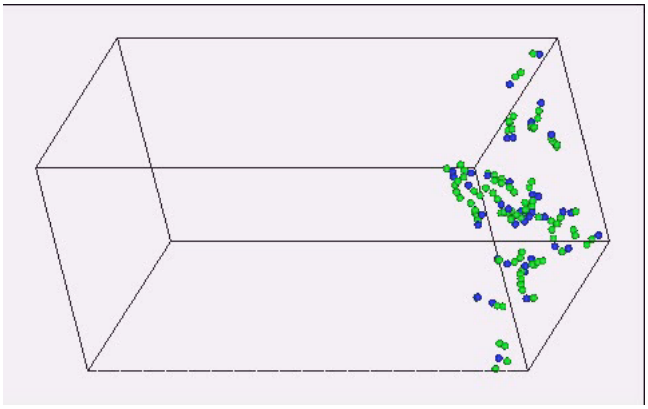
F.Soo and J.A. Theriot , PNAS, 102, 45, 16233 (2005)



Where do nanoscale displacement fluctuations come from?

What is the role of adhesion?

Simulations: Formation of Comet Tail



Actin monomers

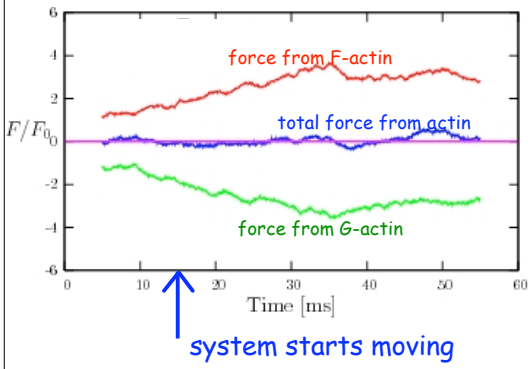
Minus ends

Branch points

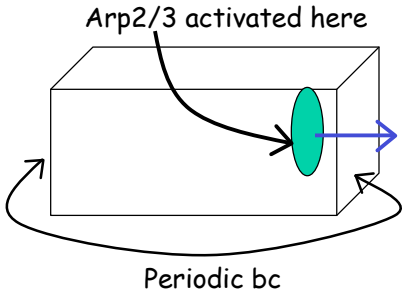
Formation of actin comet tail

(Free monomers not shown)

Force vs. Time During Onset of Motion

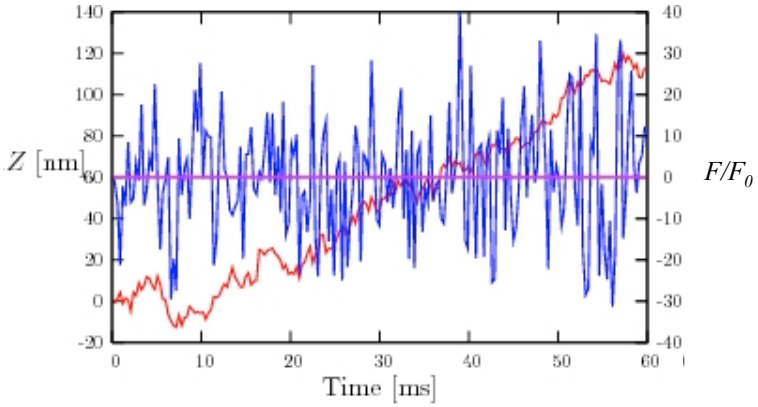


system starts moving



- 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 $\langle F_F \rangle / F_0 = 3.12$

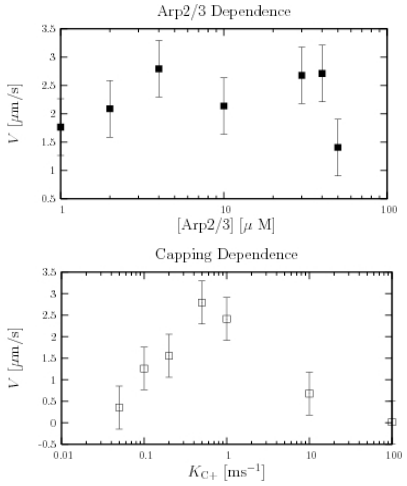
Av. force from monomers $\langle F_G \rangle / F_0 = -2.97$

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

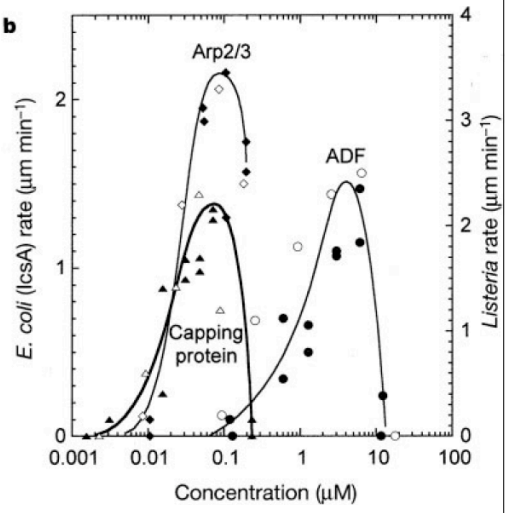
Fluctuations in force are **enormous** compared to average

Branching/Capping Protein Dependence

Simulation results

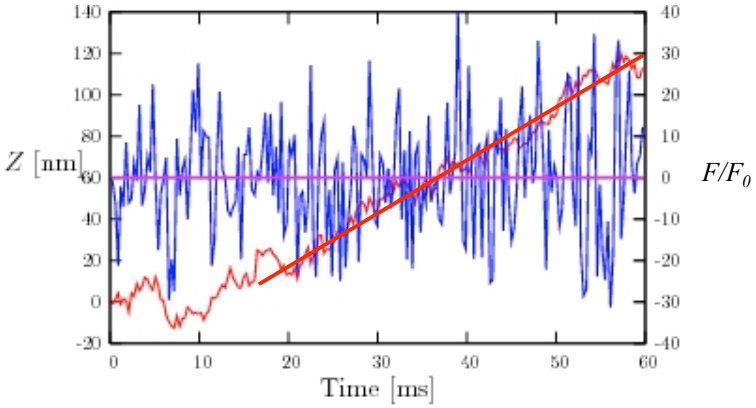


Reasonable agreement with expt



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

Nature of Motion



- Speed is approx 1μm/s (no adhesion)
- Nanoscale displacement fluctuations even without adhesion to surface

Origin of Displacement Fluctuations

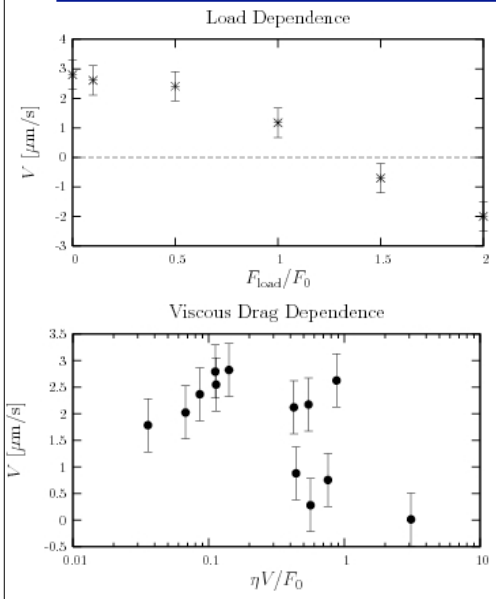
Dynamical Processes (with capping)

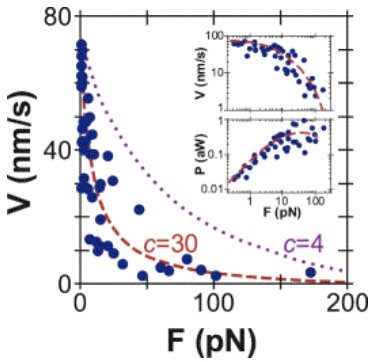
- high [capped plus ends] $\Longrightarrow$ more [branches]
- high [branches] $\Longrightarrow$ more [plus ends]
- high [plus ends] $\Longrightarrow$ more [capped plus ends]

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

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

Speed vs. Load





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

Simulations stall at much smaller loads: effect of adhesion?

Summary (Part II)

- 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 in open system; 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
- Keeping explicit monomers is crucial

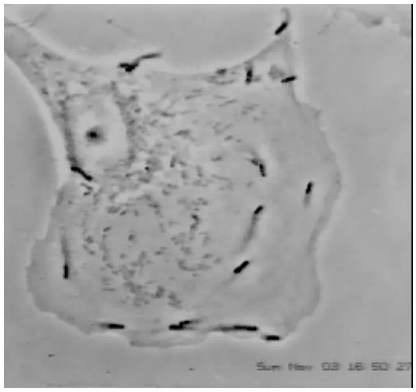
Open Questions

- What happens when filaments can adhere to the surface?
- Is there an optimal F-actin flexibility for motility?
- Why is the branching angle 70°?

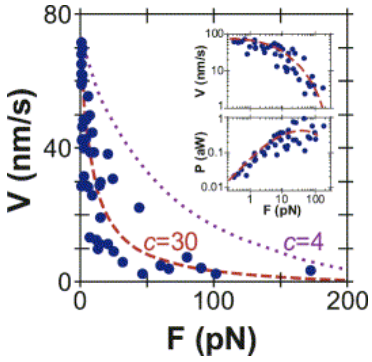
Kun-Chun Lee (U. Penn)

Motility with Surface Adhesion

x 150



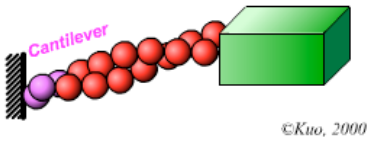
Courtesy of Julie Theriot, http://cmgm.stanford.edu/theriot/

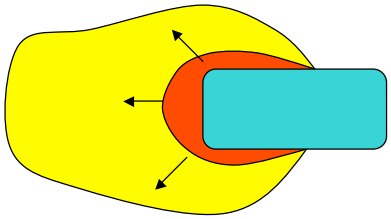


J. L. McGrath, et al. Current Biology, 13, 329-332 (2003).

- Bacterium plows through cell at nearly constant speed, despite large differences in local viscosity (load)
- Could this be due to adhesion to surface?

Some Previous Models





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

Working vs. attached filaments

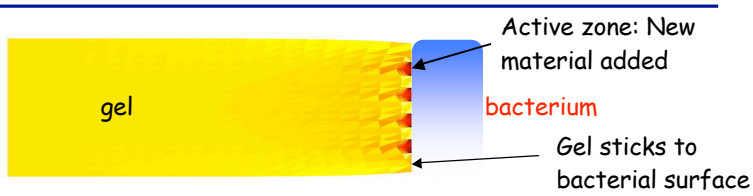
BUT these are coupled together via actin gel structure

Macroscopic models

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

BUT no nanoscale fluctuations; stress buildup generated by surface curvature

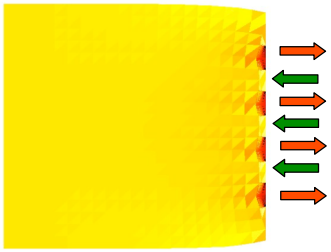
Our Model (Ajay Gopinathan)



- New material is added at active zones; polymerization rate depends on local stress, strain
- 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.)

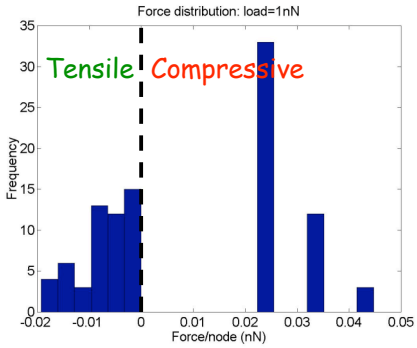
*Object Oriented Finite Element Analysis for Real Material Microstructures

Inhomogeneous Stress Distribution

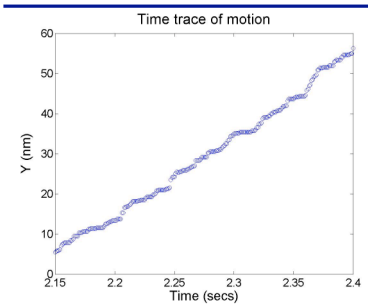


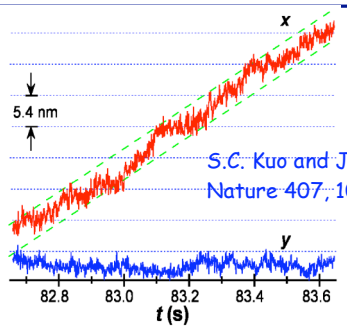
Gel surface under compressive stress so force is forwards →

Gel surface under tensile stress so force is backwards ←



Nature of Motion

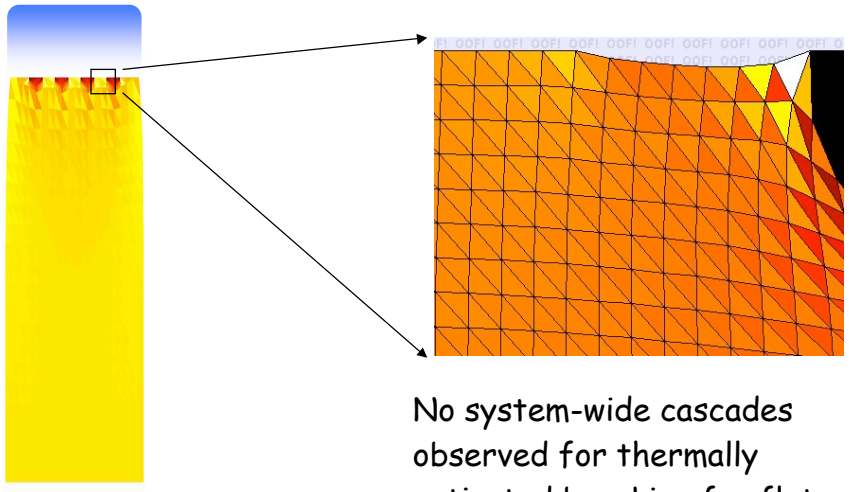




S.C. Kuo and J.L. McGrath, Nature 407, 1026 (2000)

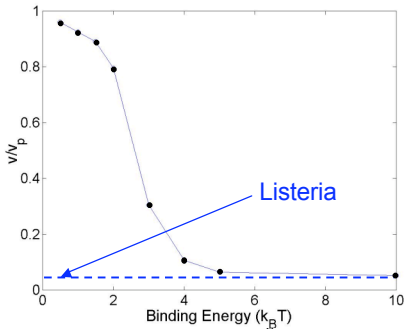
- We see nanoscale displacement fluctuations in the motion
  - Size of fluctuation depends on gel modulus, adhesion energy and mesh size
- This has nothing to do with the monomer size since our model does not contain monomers

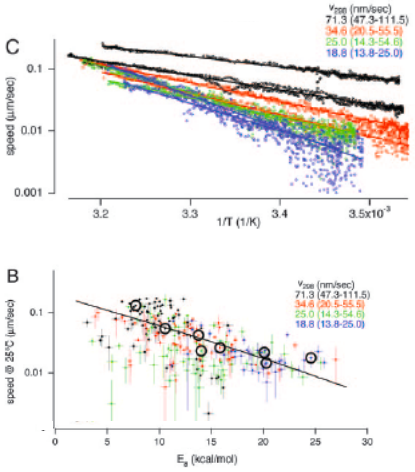
Why Steps? Adhesive Failure Cascades



No system-wide cascades observed for thermally activated breaking for flat surfaces

Speed vs. Binding Energy

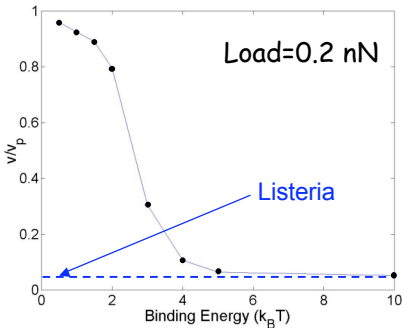


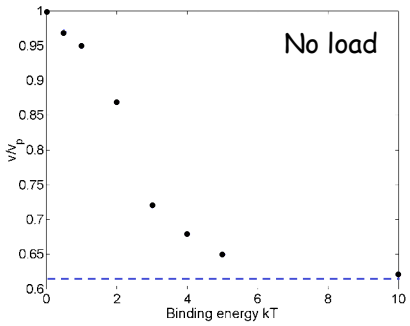


- Speed drops rapidly for small binding energies
- But it is insensitive to large binding energies

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

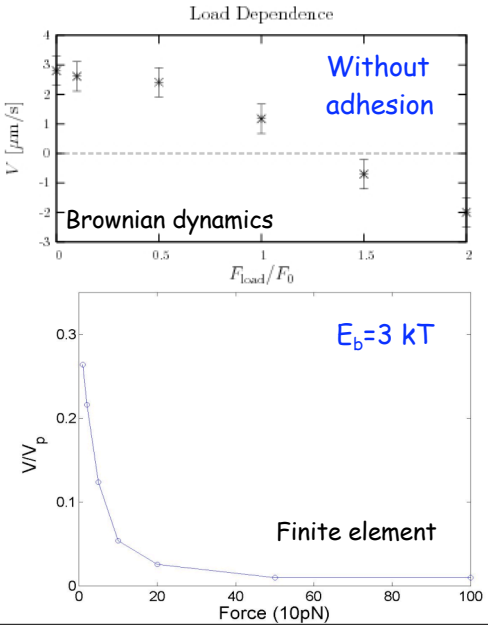
Speed vs. Binding Energy

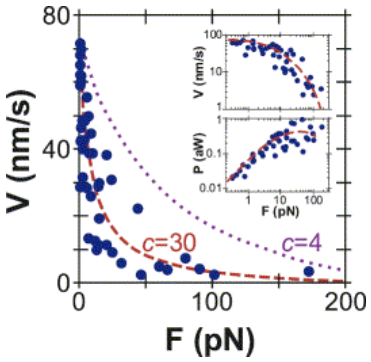




- Speed at large binding energies depends on load
- As load increases, speed decreases at large binding energies

Speed vs. Load

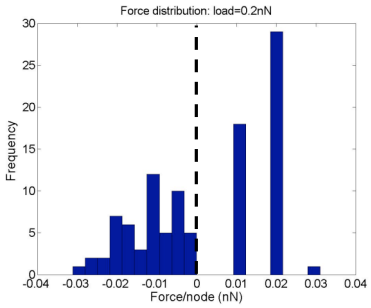


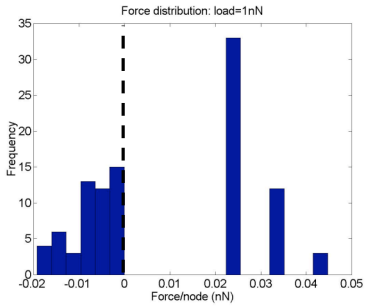


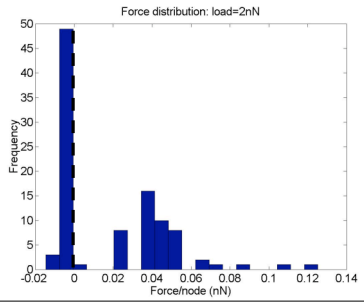
J. L. McGrath, et al. Current Biology, 13, 329-332 (2003).

Adhesion is necessary to achieve constant speed at high loads

Force Distributions vs. Load







- Average compressive force increases with load
- Average tensile force decreases slightly with increasing load
- More small tensile forces at higher loads--frequent breaking

Summary (Part III)

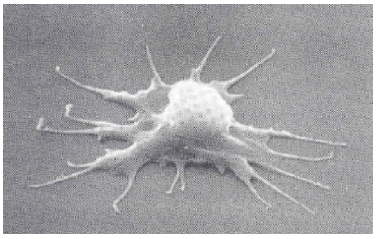
- Dynamic gel picture appears to capture many features of Listeria motility
  - Nanoscale displacement fluctuations
  - Adhesion-controlled motility
  - Force-speed relationship
- working and attached filaments are coupled
- no special geometry of bacterium is required

Ajay Gopinathan(UCSB)

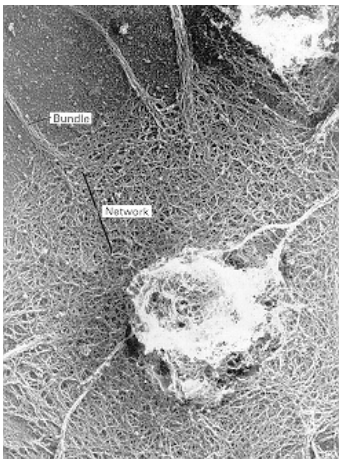
Filopodia vs. Networks

Blood platelets in resting state

Itamar Borukhov, R. F. Bruinsma, W. M. Gelbart



Activated platelet

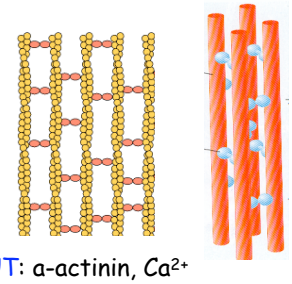


Molecular Cell Biology Lodish, et al.
J. Hartwig

Actin Binding Proteins

Bundling Proteins

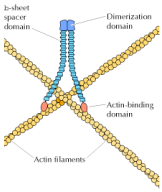
Villin, fimbrin, forked, fascin, espin,plastin, quail, scruin, …

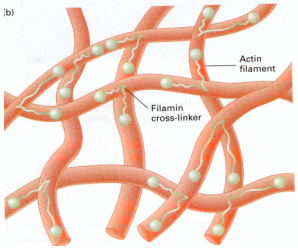


BUT: a-actinin, Ca^{2+} at high concentrations

Crosslinking Proteins

a-actinin, spectrin, dystrophin, filamin, …



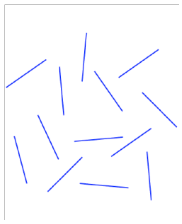
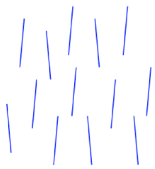
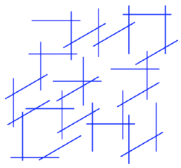
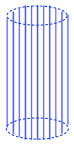


BUT: fascin, Ca^{2+} at low concentrations

- Textbook argument: protein structure determines function
- BUT: question of **concentration**

Many-Chain Phase Behavior

- What are the different possible phases for charged rigid rods with linkers?

Isotropic	Nematic	Cubatic	Bundle
			
Favored by orientational entropy	Favored by excluded volume repulsion	Favored by elec. repulsion	Favored by linker-mediated attraction

Isotropic-Nematic Transition (Onsager)

Consider hard rigid rods with length L >> diameter D

No attractions!

$$\beta F = \underbrace{c_r(\ln c_r - 1)}_{\text{translational entropy}} + \underbrace{\sigma(\{f\})c_r}_{\text{rotational entropy}} + \underbrace{\frac{1}{2}w(\{f\})c_r^{\,2}}_{\text{excluded volume}}$$

Rotational entropy: $$\sigma(\{f\}) = \int d\Omega f(\Omega)\ln[4\pi f(\Omega)]$$

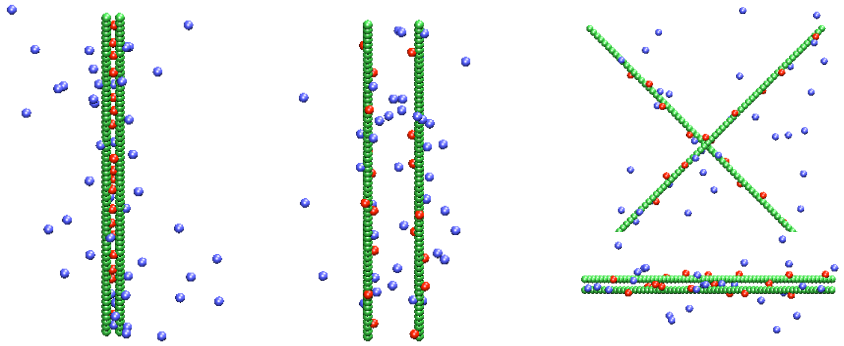
Excluded volume: $$w(\{f\}) = \int d\Omega_1 f(\Omega_1)\int d\Omega_2 f(\Omega_2)B(\gamma_{12})$$

$$B(\gamma) = \int dr\left(1 - e^{-\beta v(r,\gamma)}\right) \approx DL^2|\sin\gamma|$$

First order phase transition at volume fraction $\phi^* \approx D/L$

What is Linker-Mediated Attraction?

Molecular Dynamics Simulations (K. C. Lee, I. Borukhov, W. M. Gelbart, A. J. Liu, M. J. Stevens, Phys. Rev. Lett. **93**, 128101 (2004).)

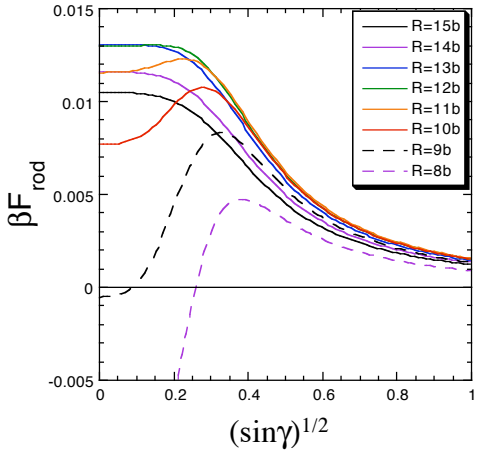


$r=2.1\sigma, \gamma=0$ $r=8\sigma, \gamma=0$ $r=2.1\sigma, \gamma>0$

Rods are 64 monomers long, neutralized by a mixture of +1 and +3 counterions

+3 ions can be **linkers** but +1 ions cannot

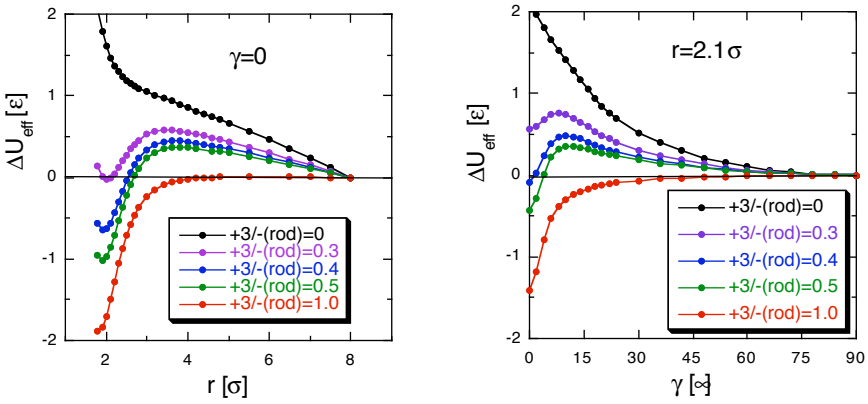
Theoretical Prediction



Rods fully neutralized by multivalent ions

B. -Y. Ha and A. J. Liu, Europhys. Lett. 46, 624 (1999).

Linker-Mediated Rod-Rod Interaction

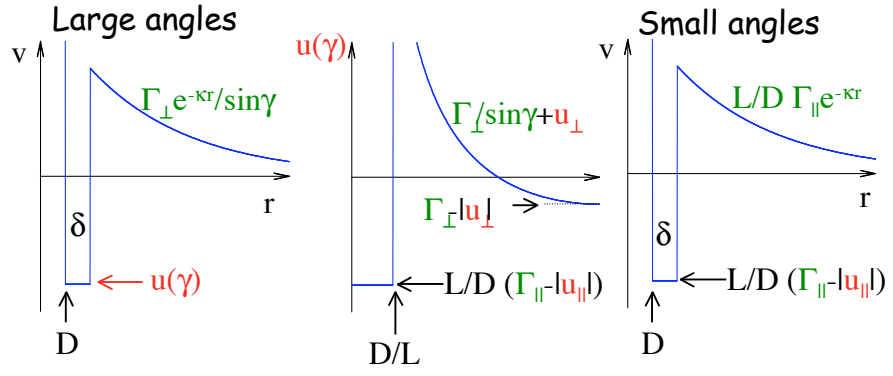


$$\Delta U_{\text{eff}}(r,\gamma=0) \int -\int_{8\sigma}^{r} f_r(r',\gamma=0)dr' \qquad \Delta U_{\text{eff}}(r=2.1\sigma,\gamma) = -\int_{\pi/2}^{\gamma} \tau(r=2.1\sigma,\gamma')d\gamma'$$

Arbitrary reference points at $r = 8\sigma$ and $\gamma = 90°$

K. C. Lee, I. Borukhov, W. M. Gelbart, A. J. Liu, M. J. Stevens, Phys. Rev. Lett. **93**, 128101 (2004).

Model Rod-Rod Interaction



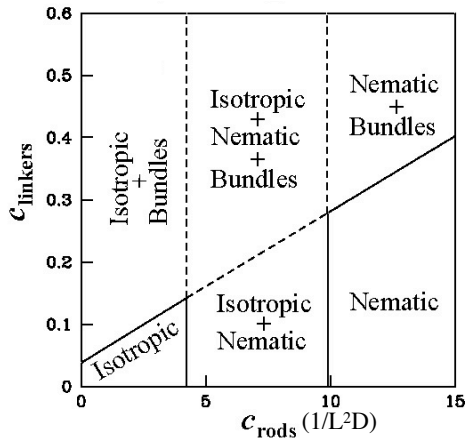
- Electrostatic repulsion: $\Gamma_{\perp}\ \Gamma_{||} > 0$
- Linker-mediated attraction: $u_{\perp}\ u_{||} < 0$

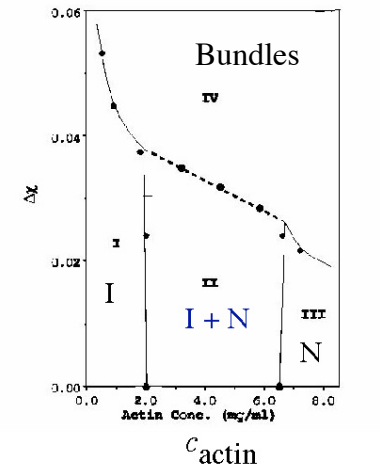
$$\frac{L}{D}u_{\|} = -n_{\|}\ln\left[1+e^{\mu_\ell - 2\varepsilon_{\|}}\right] + 2n_{\|}\ln\left[1+e^{\mu_\ell-\varepsilon_{\|}}\right] \qquad u_{\perp} = -n_{\perp}\ln\left[1+e^{\mu_\ell-2\varepsilon_{\perp}}\right] + 2n_{\perp}\ln\left[1+e^{\mu_\ell-\varepsilon_{\perp}}\right]$$

- Phenomenological quantities can be extracted from MD

Phase Diagram I

(I) Bundle-Dominated Case





$\beta\Gamma_{\parallel} = \beta\Gamma_{\perp} = 1.26, \beta\varepsilon_{\parallel} = \beta\varepsilon_{\perp} = -7$

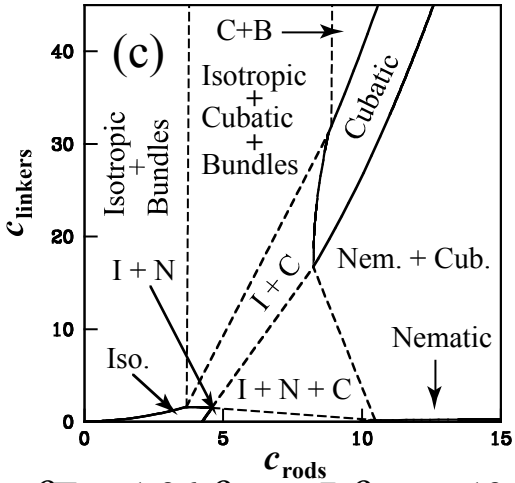
I. Borukhov, R. F. Bruinsma, W. M. Gelbart, A. J. Liu, PNAS **102**, 3673 (2005).

F-Actin with Depletion Attraction

Suzuki, Yamazaki, Ito, Biochem **35**, 5238 (1996).

Phase Diagram II

(II) Networks and Bundles

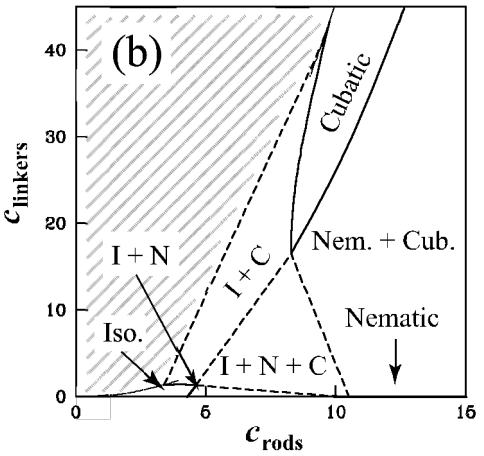


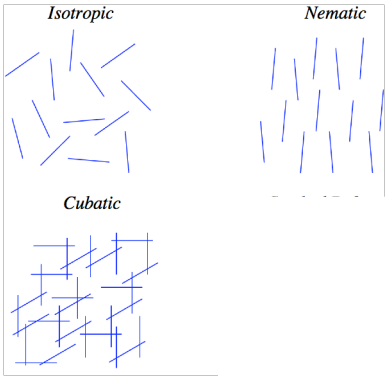
$\beta\Gamma_{\parallel} = \beta\Gamma_{\perp} = 1.26; \beta\varepsilon_{\parallel} = -7, \beta\varepsilon_{\perp} = -10.5$

I. Borukhov, R. F. Bruinsma, W. M. Gelbart, A. J. Liu, PNAS 102, 3673 (2005).

Phase Diagram III

(III) Network-Dominated Case

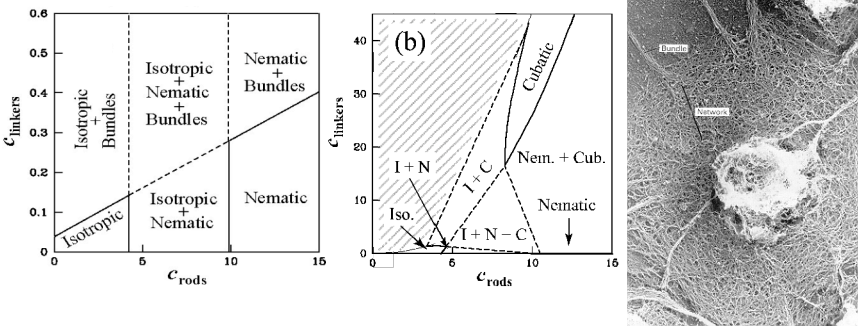




$$\beta\Gamma_{\parallel} = \beta\Gamma_{\perp} = 1.26; \beta\varepsilon_{\parallel} = -7, \beta\varepsilon_{\perp} = -12$$

I. Borukhov, R. F. Bruinsma, W. M. Gelbart, A. J. Liu, PNAS 102, 3673 (2005).

Possible Connection to Cytoskeleton



- Jump from bundle-dominated to network-dominated diagram in < 1 $k_B T$
- VASP changes binding energy by phosphorylation
- Actin polymerization and depolymerization can take system around kinetic barriers--no need for spatial gradients

Summary (Part IV)

- Small differences in linker binding can lead to very different morphologies for long filaments

  Networks vs. Bundles

- This may be relevant to structure at the leading edge of a crawling cell
- Proximity to a phase transition is one way of achieving high sensitivity in biological systems

Itamar Borukhov (Compugen)