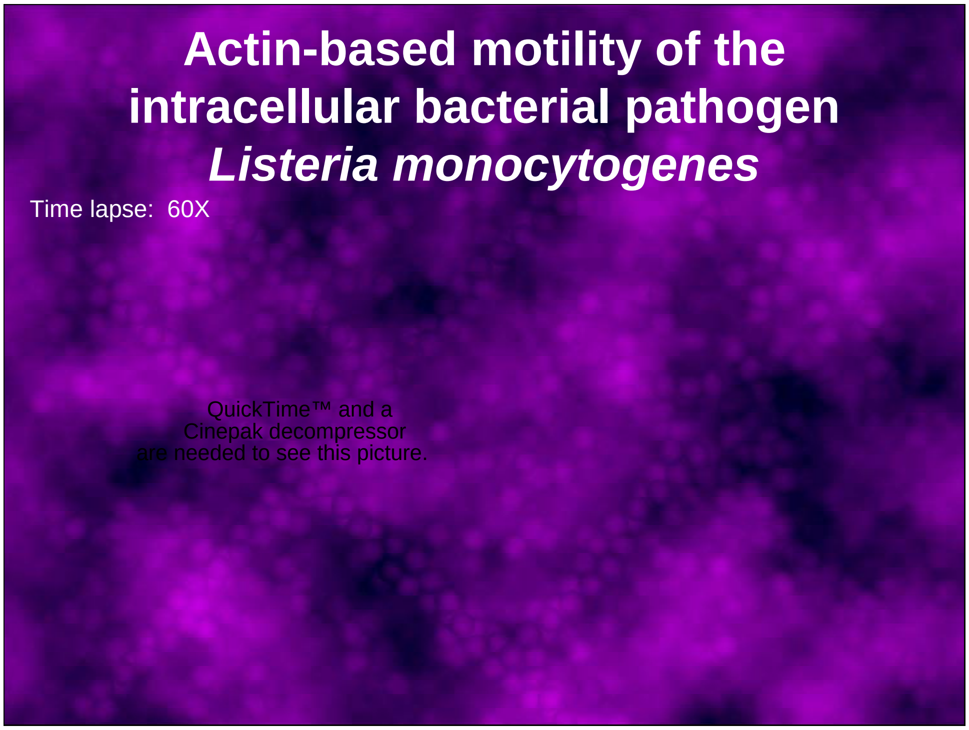


Speeded up ?X. PtK2 cells. Note the beautiful lamella, by which the cell undergoes it's own actin-based motility.

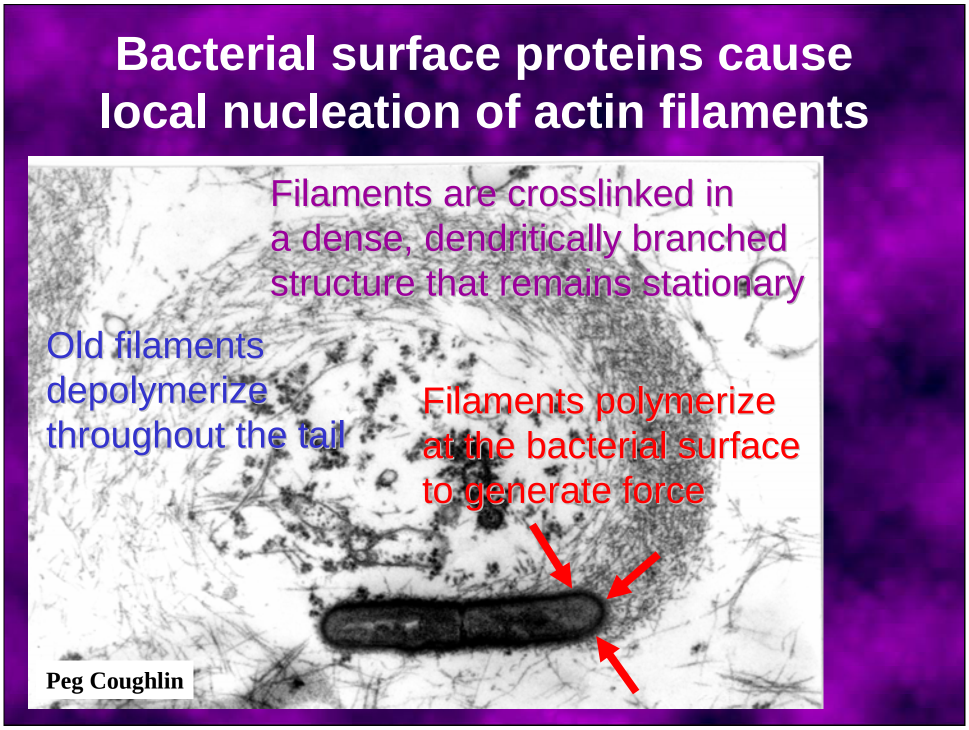
Gram positive bacillus

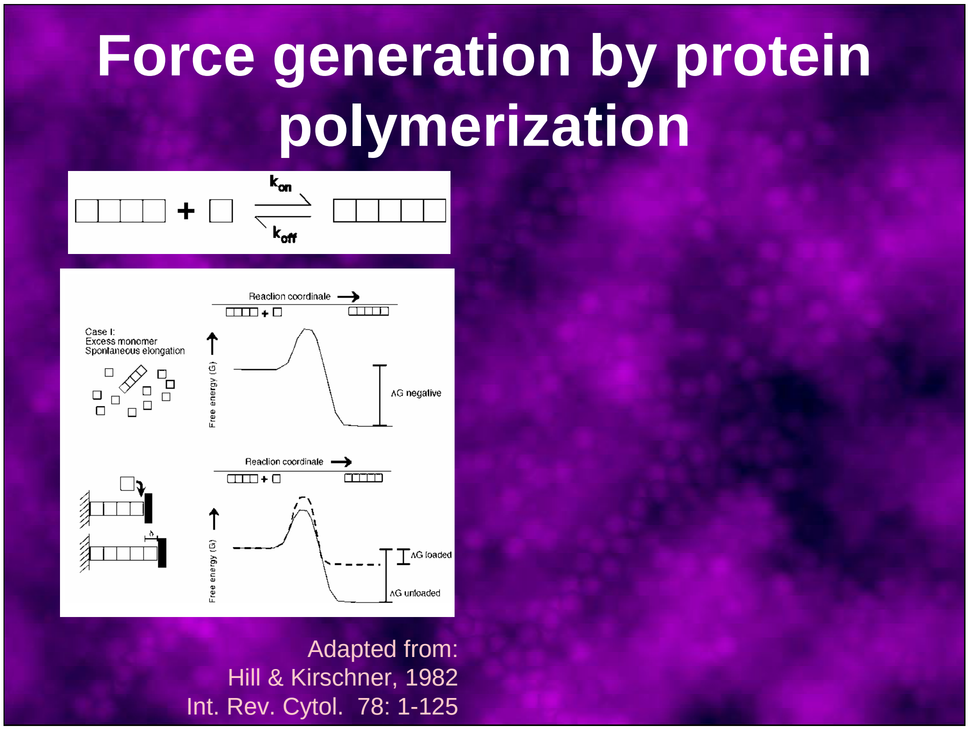
Propelled through the cytoplasm via actin polymerization

10 different labs working this out I do next step

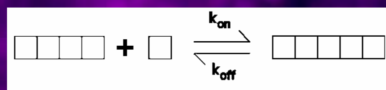
Other organisms which use this form of motility, beautiful example of coevolution. Shigella, Rickettsia, Vaccinia

Why evolve this motile mechanism? To run from the immune system.

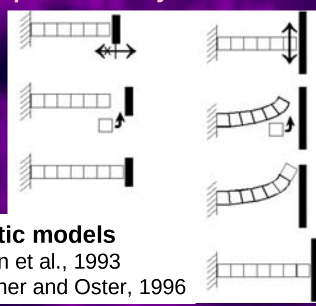

Bacterial surface proteins cause local nucleation of actin filaments
Filaments are crosslinked in a dense, dendritically branched structure that remains stationary
Old filaments depolymerize throughout the tail
Filaments polymerize at the bacterial surface to generate force
Peg Coughlin


Force generation by protein polymerization
kon
koff
Reaclion coordinale
Case I:
Excess monomer
Spontaneous elongation
Free energy (G)
ΔG negative
Reaclion coordinale
Free energy (G)
ΔG loaded
ΔG unloaded
Adapted from:
Hill & Kirschner, 1982
Int. Rev. Cytol. 78: 1-125

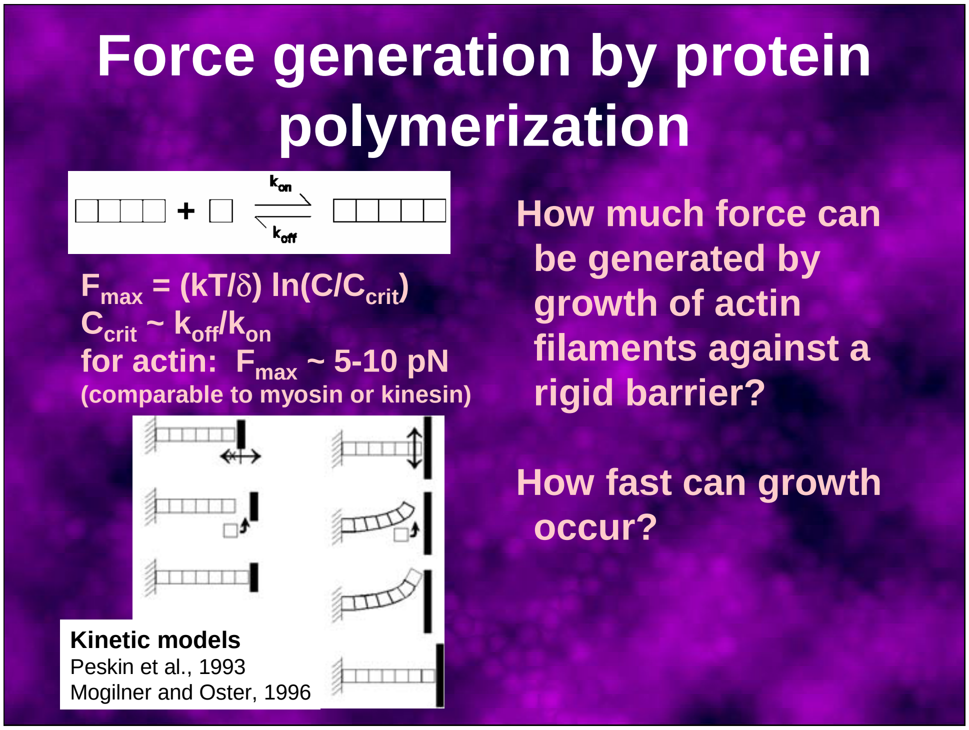
Force generation by protein polymerization



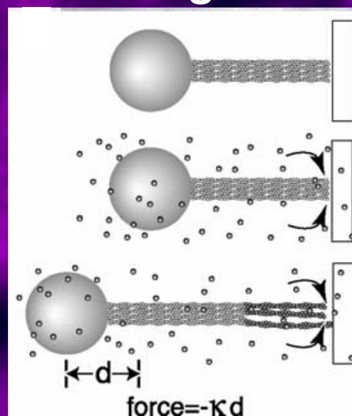
$F_{max} = (kT/\delta)\ \ln(C/C_{crit})$
$C_{crit} \sim k_{off}/k_{on}$
for actin: $F_{max} \sim$ 5-10 pN
(comparable to myosin or kinesin)



Kinetic models
Peskin et al., 1993
Mogilner and Oster, 1996

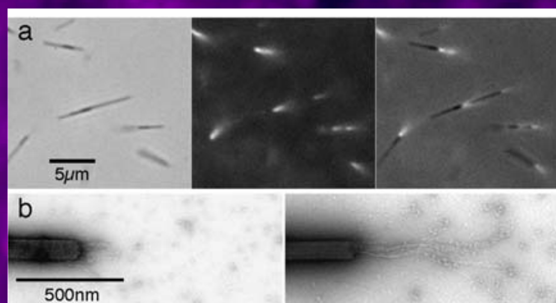

Force generation by protein polymerization
k_on
k_off
F_max = (kT/δ) ln(C/C_crit)
C_crit ~ k_off/k_on
for actin: F_max ~ 5-10 pN
(comparable to myosin or kinesin)
Kinetic models
Peskin et al., 1993
Mogilner and Oster, 1996
How much force can be generated by growth of actin filaments against a rigid barrier?
How fast can growth occur?

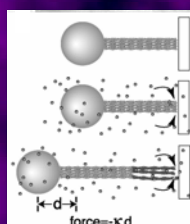
Optical trap method for measuring force from growth of a small bundle



Collaboration with:
Marileen Dogterom
Matthew Footer
Jacob Kerssemakers

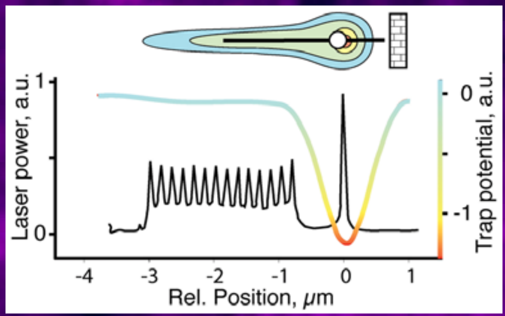
Optical trap method for measuring force from growth of a small bundle

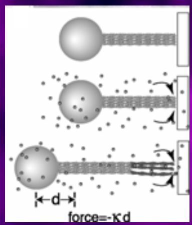




Horsehoe crab sperm acrosomal bundles nucleate actin filament growth

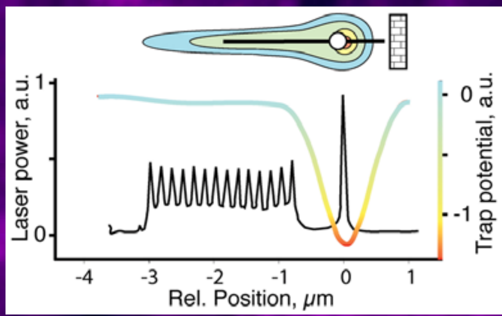
Optical trap method for measuring force from growth of a small bundle

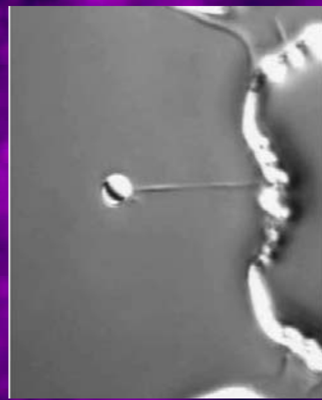


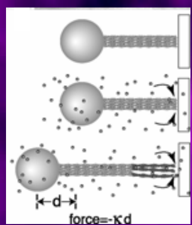


Keyhole optical trap holds bead and bundle next to wall

Optical trap method for measuring force from growth of a small bundle

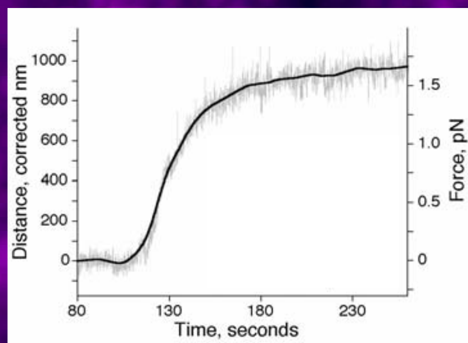


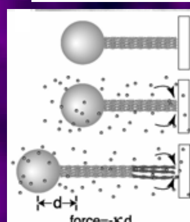




Keyhole optical trap holds bead and bundle next to wall

Growth slows to stall at a few pN





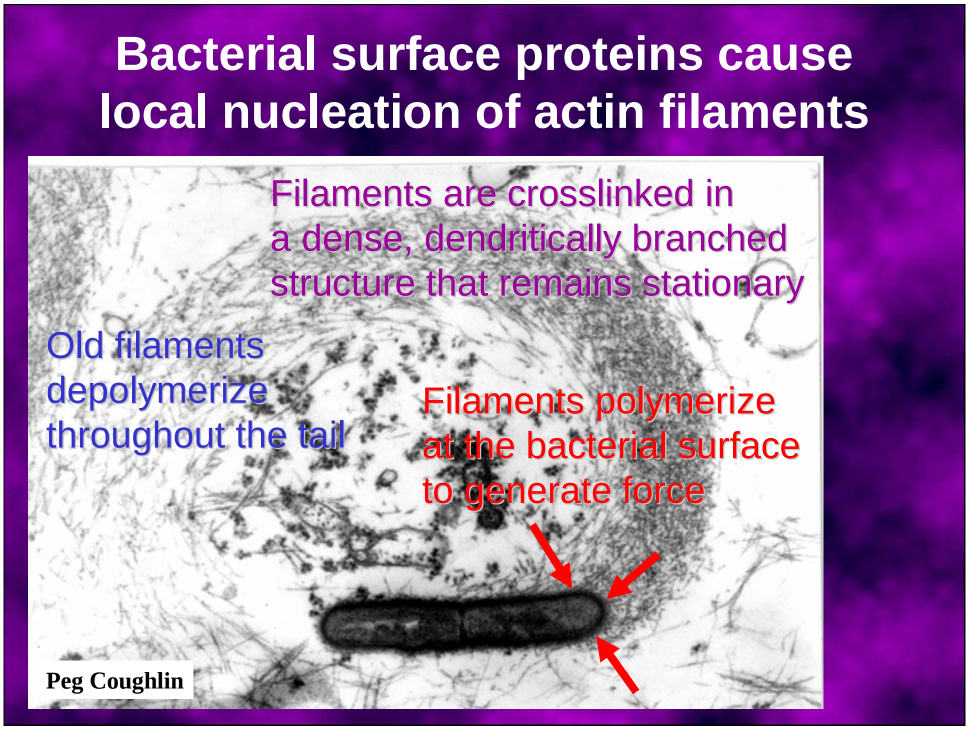
Growth condition:
4 µM actin
20 µM profilin

Stall occurs near
Hill & Kirschner limit

Building up in scale

Individual filaments can generate a few picoNewtons of force; small bundles are not able to work together efficiently to push harder

Are branched networks better generators of polymerization-driven pushing force than bundles?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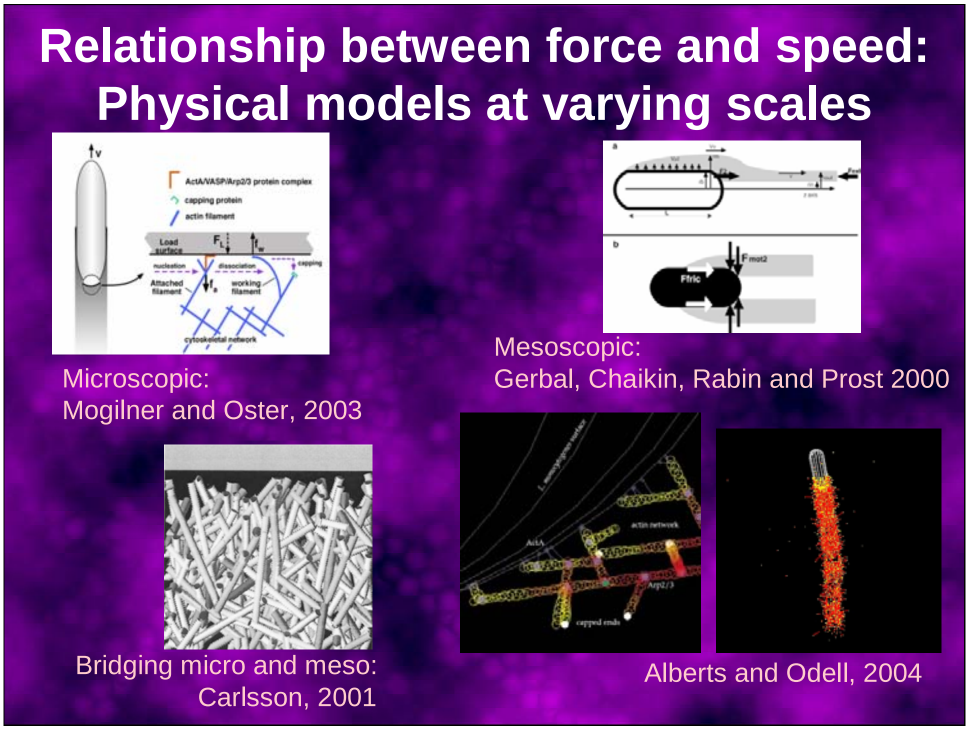
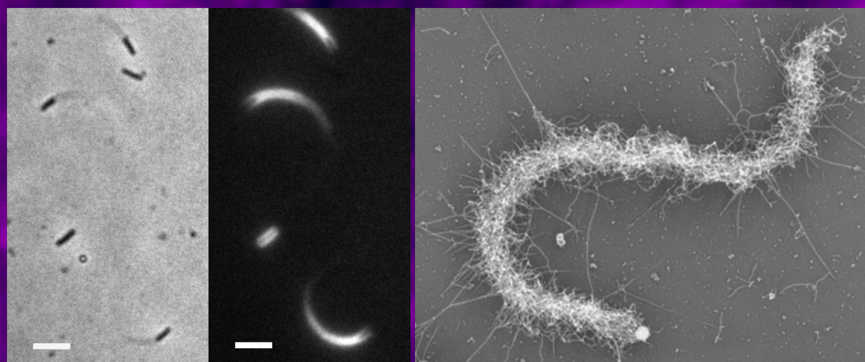
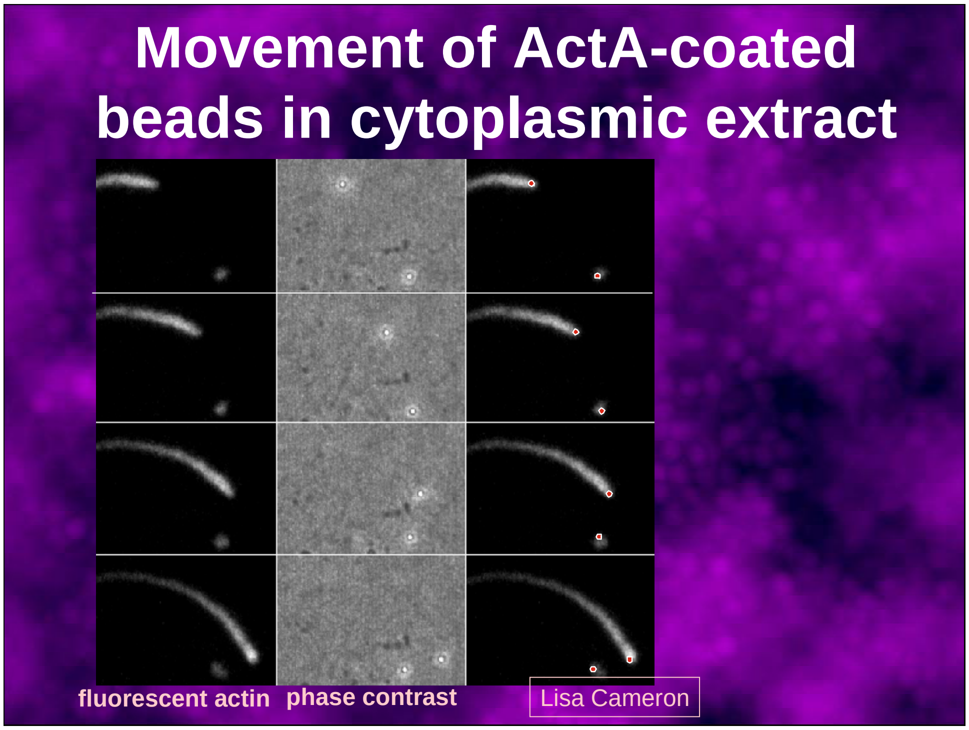

Relationship between force and speed: Physical models at varying scales
ActA/VASP/Arp2/3 protein complex
capping protein
actin filament
Load surface
nucleation
dissociation
capping
Attached filament
working filament
cytoskeletal network
Microscopic:
Mogilner and Oster, 2003
Mesoscopic:
Gerbal, Chaikin, Rabin and Prost 2000
Bridging micro and meso:
Carlsson, 2001
actin network
ActA
Arp2/3
capped ends
Alberts and Odell, 2004

Biochemical and biophysical manipulations of actin comet tails



Movement in cytoplasmic extracts
(Theriot et al., 1994)
Reconstitution with purified proteins
(Loisel et al., 1999)

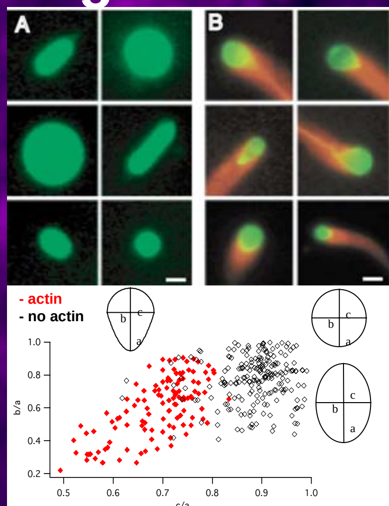
Replacement of bacteria by
ActA-coated polystyrene beads
(Cameron et al., 1999)


Movement of ActA-coated beads in cytoplasmic extract
fluorescent actin
phase contrast
Lisa Cameron

Movement of ActA-coated beads in cytoplasmic extract

Lisa Cameron

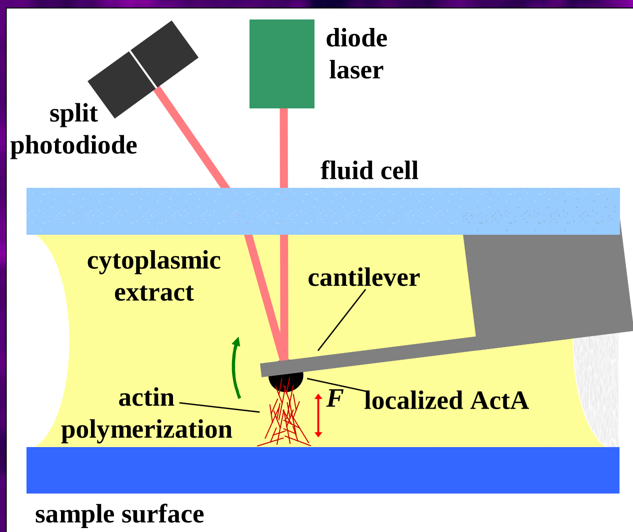
Force magnitude: 1 comet tail generates ~1 nanoNewton



Vesicle deformation shows that tails squeeze their cargo

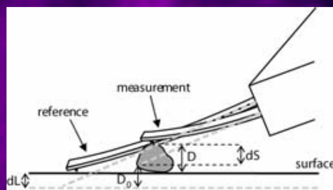
Paula Giardini

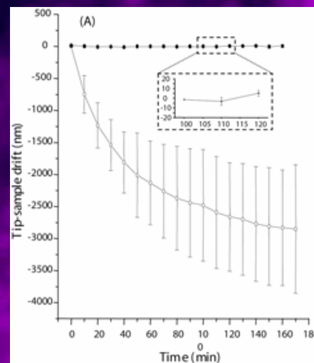
Experimental design for measurement of comet tail force using microfabricated cantilevers



Dan Fletcher

Dual-beam cantilever AFM design for measuring slow, strong network growth

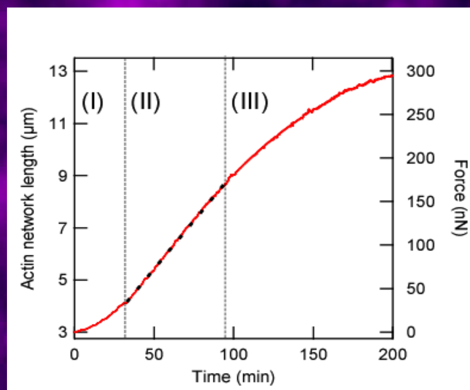




Drift over 3 hours reduced from 3 µm to a few nm

Dan Fletcher, Jason Choy

Cantilever deflection driven by actin comet tail growth



I. Acceleration

II. Constant growth

III. Slowing to stall

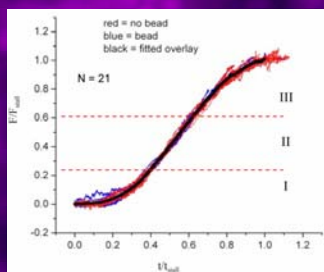
NOTE: scale is in NANONEWTONS

Dan Fletcher, Sapun Parekh

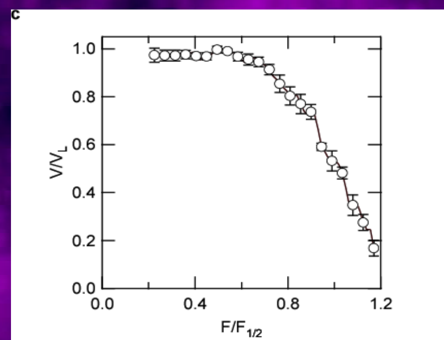
Force-velocity curve shows distinct phases

Stall force =
2.9 ± 1.3 nN/μm²

(~7 pN/filament)

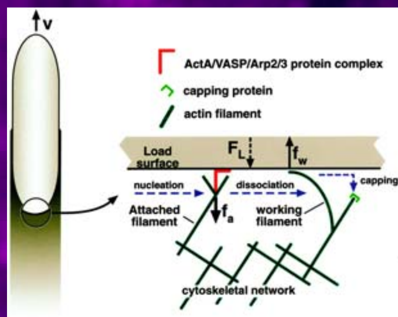


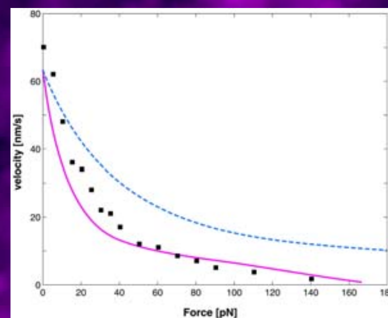
Curve shape is similar regardless of surface geometry



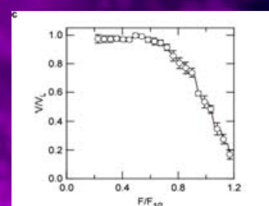
Dan Fletcher, Sapun Parekh

What did the models predict?





Mogilner and Oster, 2003
"Tethered Ratchet"
NO flat phase
Force decrease is concave up



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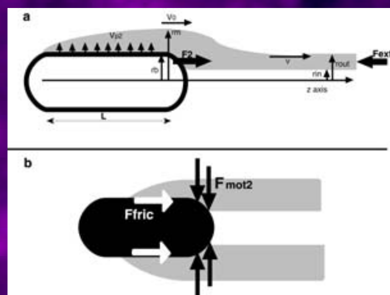
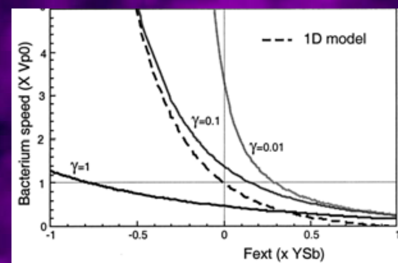
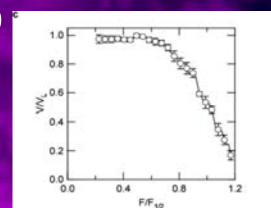




Gerbal, Chaikin, Rabin & Prost 2000
"Gel Elastic Model"
NO flat phase
Force decrease is concave up



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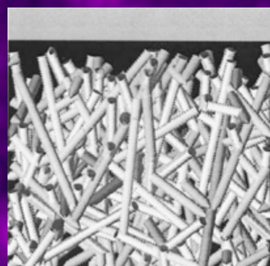
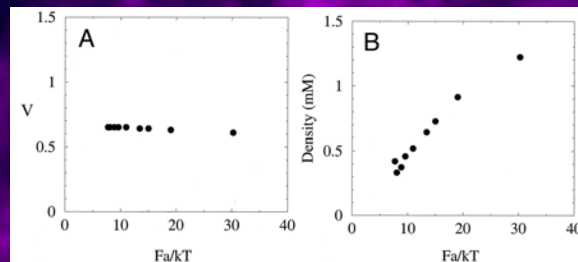
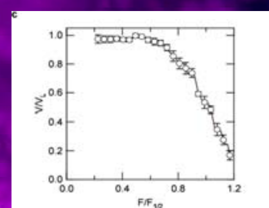


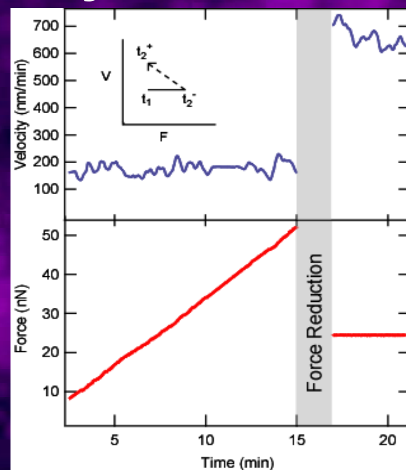


Carlsson, 2001
“Autocatalytic Nucleation”
Flat phase lasts because gel density increases!!
Not carried out to fall-off

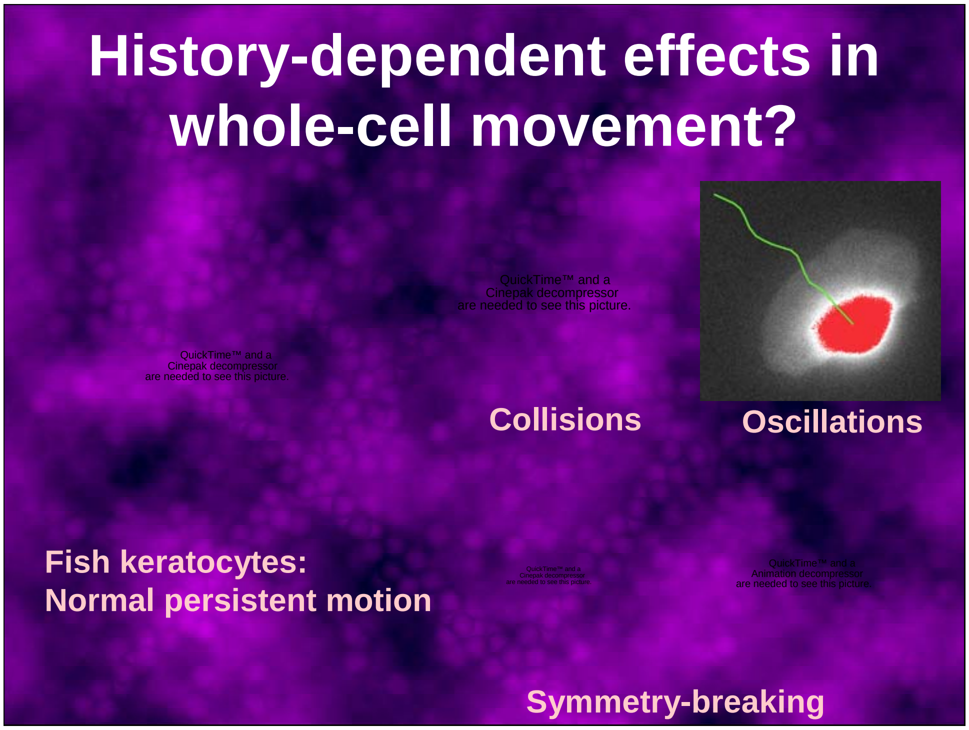


Hysteresis in the force-velocity curve

- Force-loading to pre-stress gel may increase filament density
- Restoration to lower load causes FASTER gel growth: up to 3X faster than same load without prestressing
- Conclusion: Working out makes you stronger!



Dan Fletcher, Sapun Parekh


History-dependent effects in whole-cell movement?
QuickTime™ and a Cinepak decompressor are needed to see this picture.
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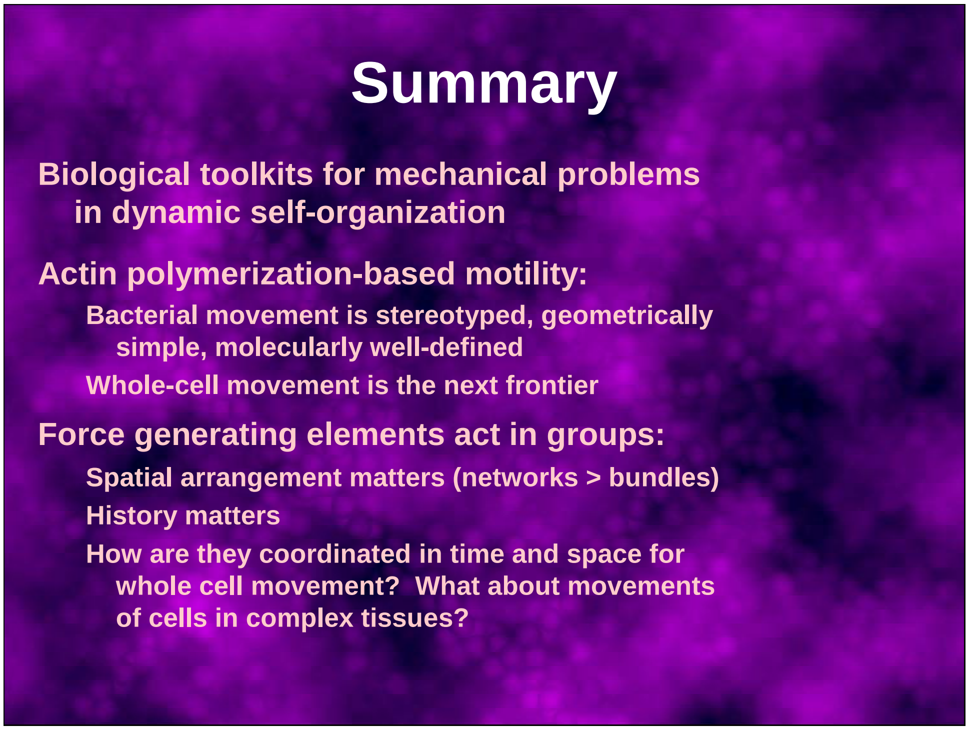
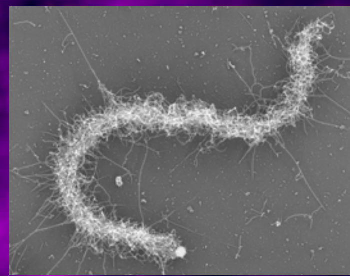
Collisions
Oscillations
Fish keratocytes:
Normal persistent motion
QuickTime™ and a Animation decompressor are needed to see this picture.
Symmetry-breaking


Summary
Biological toolkits for mechanical problems in dynamic self-organization
Actin polymerization-based motility:
Bacterial movement is stereotyped, geometrically simple, molecularly well-defined
Whole-cell movement is the next frontier
Force generating elements act in groups:
Spatial arrangement matters (networks > bundles)
History matters
How are they coordinated in time and space for whole cell movement? What about movements of cells in complex tissues?

Acknowledgements

Current:
Greg Allen, Erin Barnhart, Annie Brotcke, Natalie Dye, Aretha Fiebig, Matthew Footer, Karine Gibbs, Kinneret Keren, Catherine Lacayo, Peter Lee, Zach Pincus, Cyrus Wilson, Patricia Yam



Recent:
David Baldwin, Lisa Cameron, Martijn van Duijn, Dan Fletcher, Paula Giardini, Susanne Rafelski, Rachael Ream, Jennifer Robbins, Fred Soo, Alexander van Oudenaarden

Collaborators:
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Dan Fletcher, Jason Choy, Allen Liu, Sapun Parekh (UC Berkeley)
Manuel Amieva, Glen Otto, Mickey Pentecost (Stanford)
Tom Silhavy, Dan Isaac (Princeton)
Roger Hendrix, Jun Xu (U. Pittsburgh)
Alex Mogilner Boris Rubenstein (UC Davis)
Paul Wiseman, Ben Hebert (McGill)
Lin Ji, Gaudenz Danuser (Scripps)
Theresa Harper (Quantum Dots, Inc.)
Dan Portnoy , Anna Bakardjiev, Pete Lauer, Vicki Auerbuch (UC Berkeley)