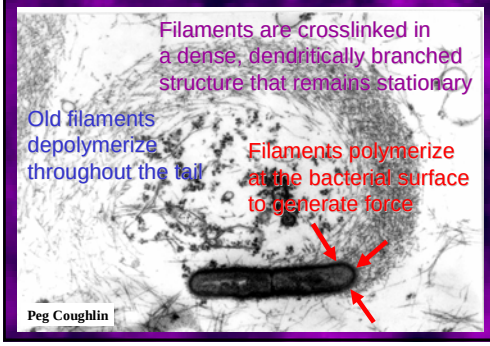
Actin-based motility of the intracellular bacterial pathogen *Listeria monocytogenes*

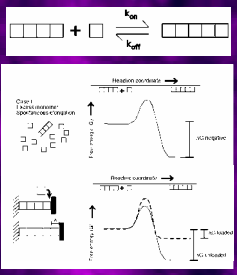
Time lapse: 60X

QuickTime™ and a Cinepak decompressor are needed to see this picture.

Bacterial surface proteins cause local nucleation of actin filaments

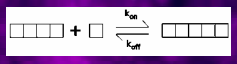


Force generation by protein polymerization

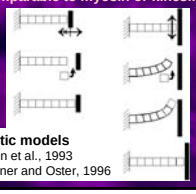


Adapted from:
Hill & Kirschner, 1982
Int. Rev. Cytol. 78: 1-125

Force generation by protein polymerization

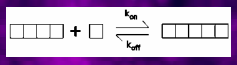


$F_{max} = (kT/\delta) \ln(C/C_{crit})$
$C_{crit} \sim k_{off}/k_{on}$
for actin: $F_{max} \sim$ 5-10 pN
(comparable to myosin or kinesin)



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

Force generation by protein polymerization



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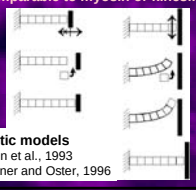
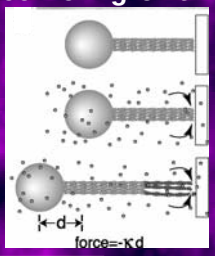


Kinetic models
Peskin et al., 1993
Mogilner and Oster, 1996

How much force can be generated by growth of actin filaments against a rigid barrier?

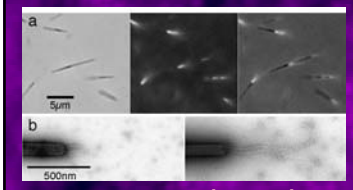
How fast can growth occur?

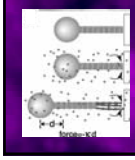
Optical trap method for measuring force from growth of a small bundle



Collaboration with:
Marileen Dogterom
Matthew Footer
Jacob Kerssemakers

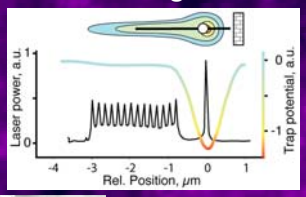
Optical trap method for measuring force from growth of a small bundle

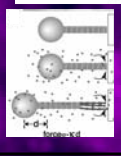




Horsehoe crab sperm acrosomal bundles nucleate actin filament growth

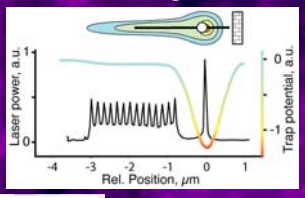
Optical trap method for measuring force from growth of a small bundle

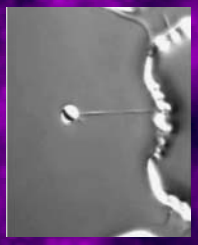


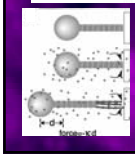


Keyhole optical trap holds bead and bundle next to wall

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

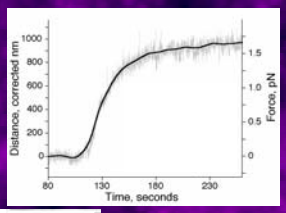


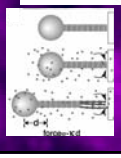




Keyhole optical trap holds bead and bundle next to wall

Growth slows to stall at a few pN





Growth condition:
4 μM actin
20 μM profilin

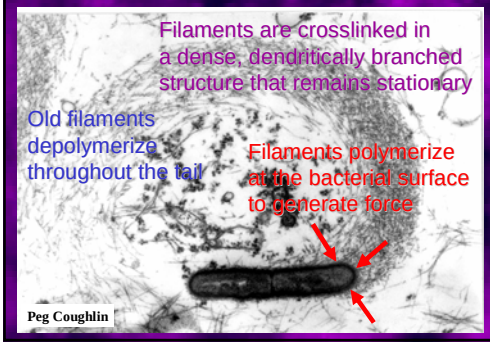
Stall occurs near
Hill & Kirschner limit

Building up in scale

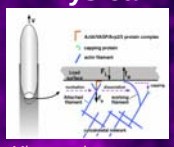
Individual filaments can generate a few picoNewtons of force; small bundles are not able to work together efficiently to push harder

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

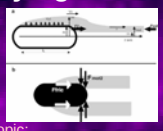
Bacterial surface proteins cause local nucleation of actin filaments



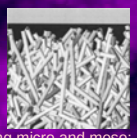
Relationship between force and speed: Physical models at varying scales



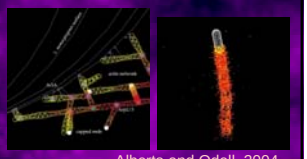
Microscopic:
Mogilner and Oster, 2003



Mesoscopic:
Gerbal, Chaikin, Rabin and Prost 2000

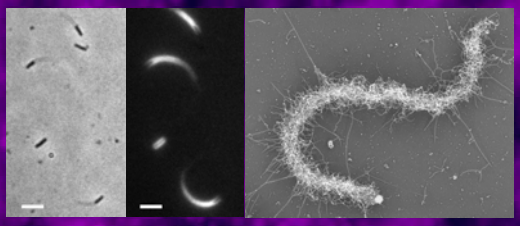


Bridging micro and meso:
Carlsson, 2001



Alberts and Odell, 2004

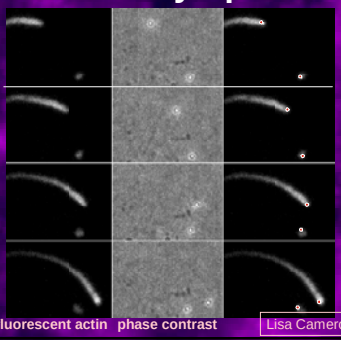
Biochemical and biophysical manipulations of actin comet tails



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



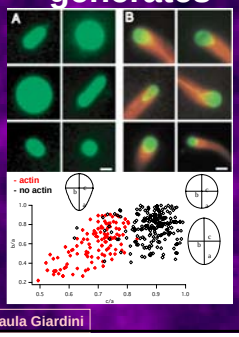
fluorescent actin phase contrast

Lisa Cameron

Movement of ActA-coated beads in cytoplasmic extract

Lisa Cameron

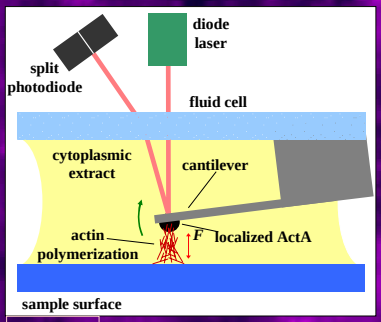
Force magnitude: 1 comet tail generates ~1 nanoNewton



Vesicle deformation shows that tails squeeze their cargo

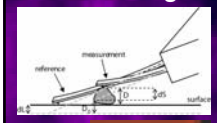
Paula Giardini

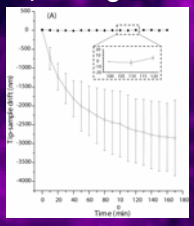
Experimental design for measurement of comet tail force using microfabricated cantilevers

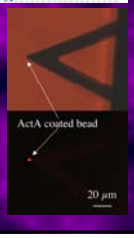


Dan Fletcher

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



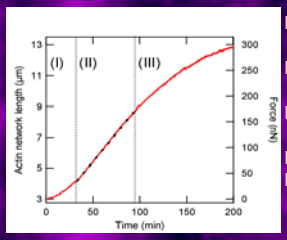




Drift over 3 hours reduced from 3 μm to a few nm

Dan Fletcher, Jason Choy

Cantilever deflection driven by actin comet tail growth



I. Acceleration

II. Constant growth

III. Slowing to stall

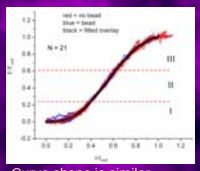
NOTE: scale is in NANONEWTONS

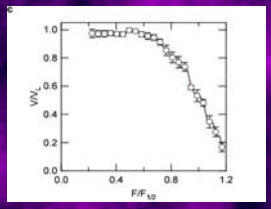
Dan Fletcher, Sapun Parekh

Force-velocity curve shows distinct phases

Stall force = 2.9 ± 1.3 nN/μm²

(~7 pN/filament)

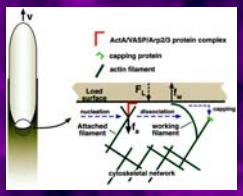


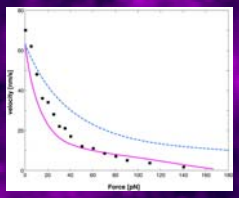


Curve shape is similar regardless of surface geometry

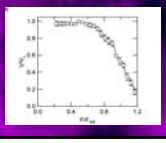
Dan Fletcher, Sapun Parekh

What did the models predict?

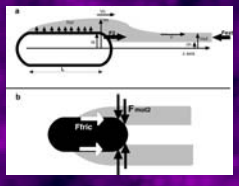


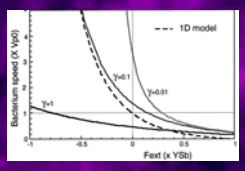


Mogilner and Oster, 2003
"Tethered Ratchet"
NO flat phase
Force decrease is concave up

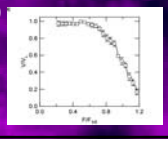


What did the models predict?





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



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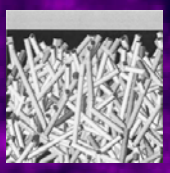
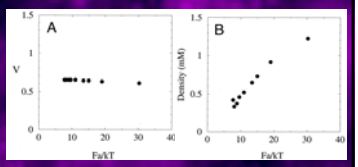
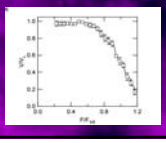


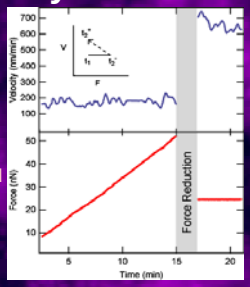


Carlsson, 2001
"Autocatalytic Nucleation"
Flat phase lasts because gel density increases!!
Not carried out to fall-off



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?

Collisions Oscillations

Fish keratocytes:
Normal persistent motion

Symmetry-breaking

Summary

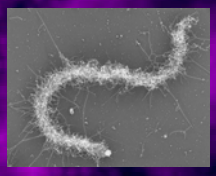
Biological toolkits for mechanical problems in dynamic self-organization

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)