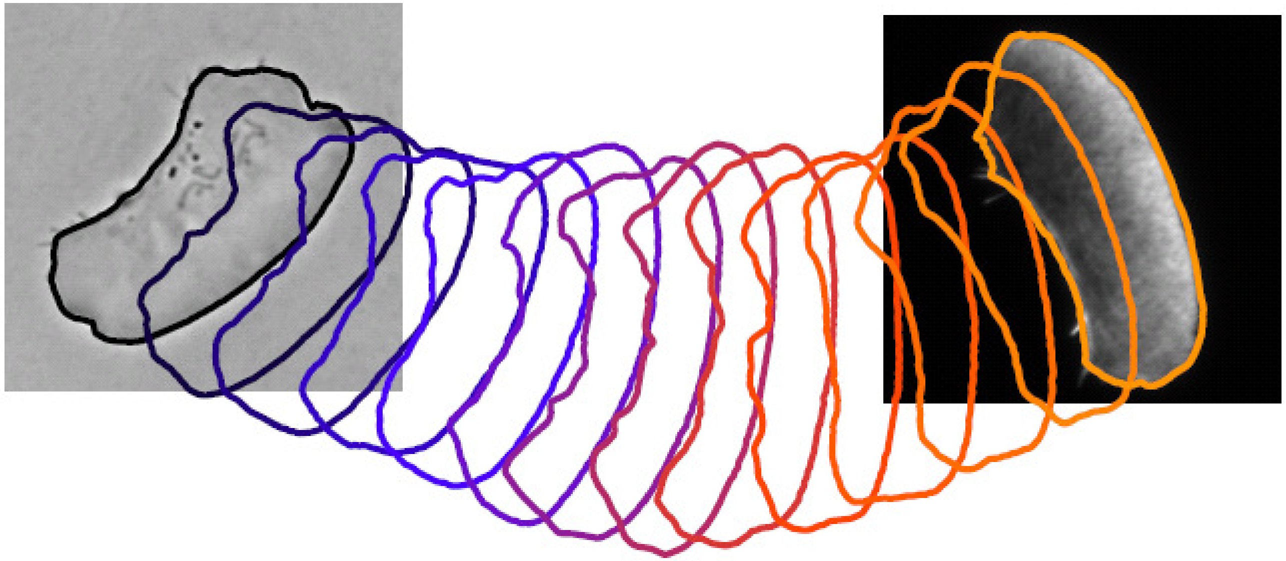
The interplay between actin dynamics and membrane tension determines the shape of moving cells



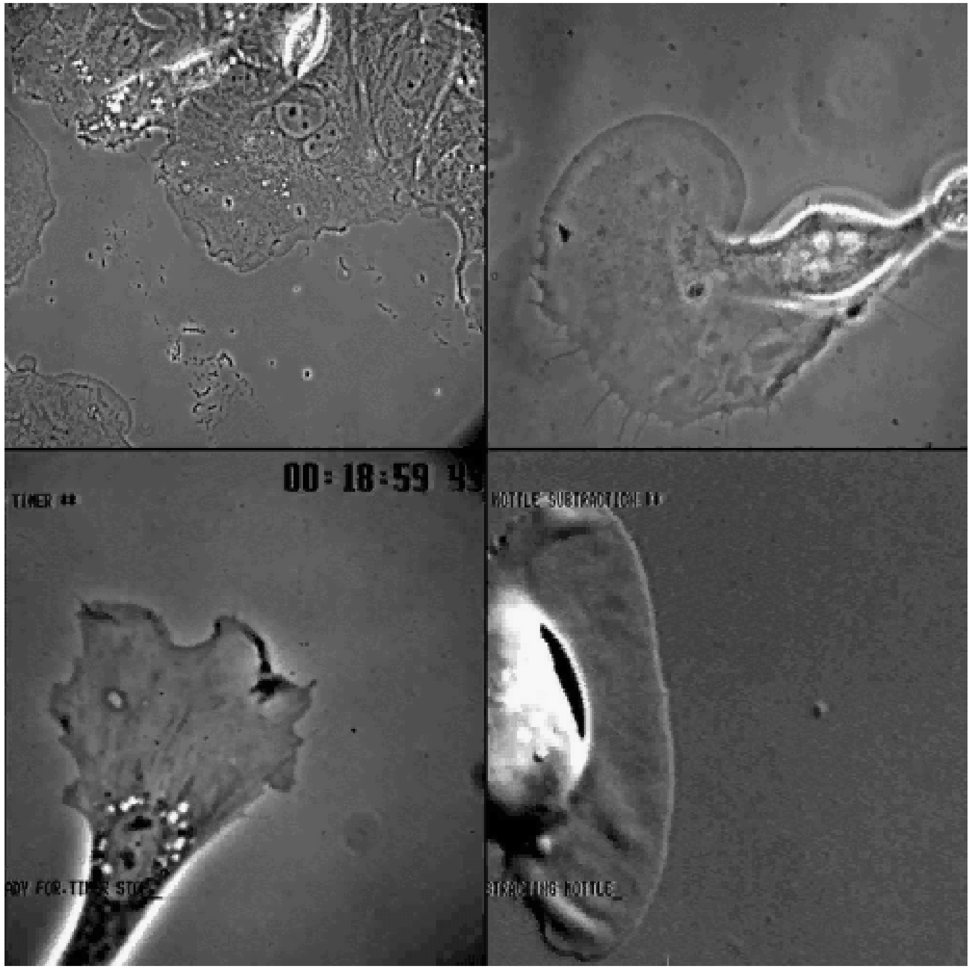
Kinneret Keren
Physics Department
Technion- Israel Institute of Technology
August, 2011

Moving cells

Cell movement is ubiquitous.
Nearly all animal cells move with the same basic mechanism: actin based motility

Mouse fibroblast
(connective tissue)
Movie duration: 3 hours

Mouse melanoma cell
Movie duration: 20 minutes



Chick fibroblast
Movie duration: 2 hours

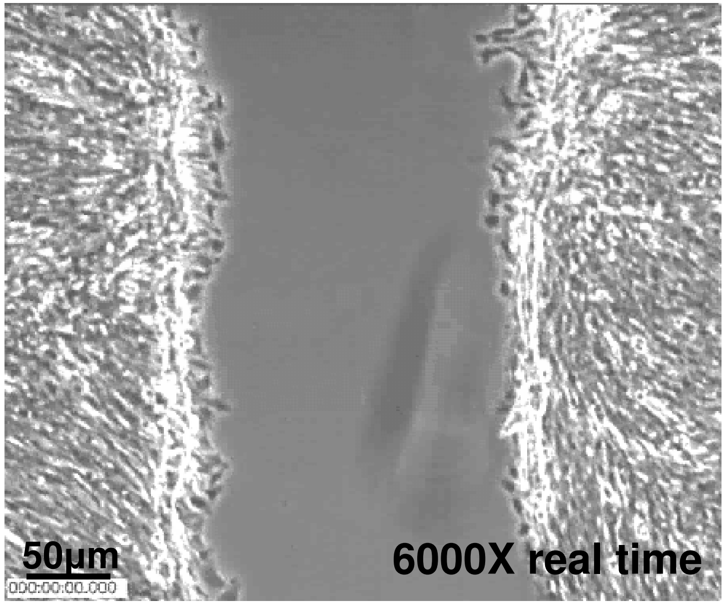
Fish keratocyte (skin)
Movie duration: 4 minutes

Movie from Vic Small
“Video tour of cell motility”

Cell movement has important functions

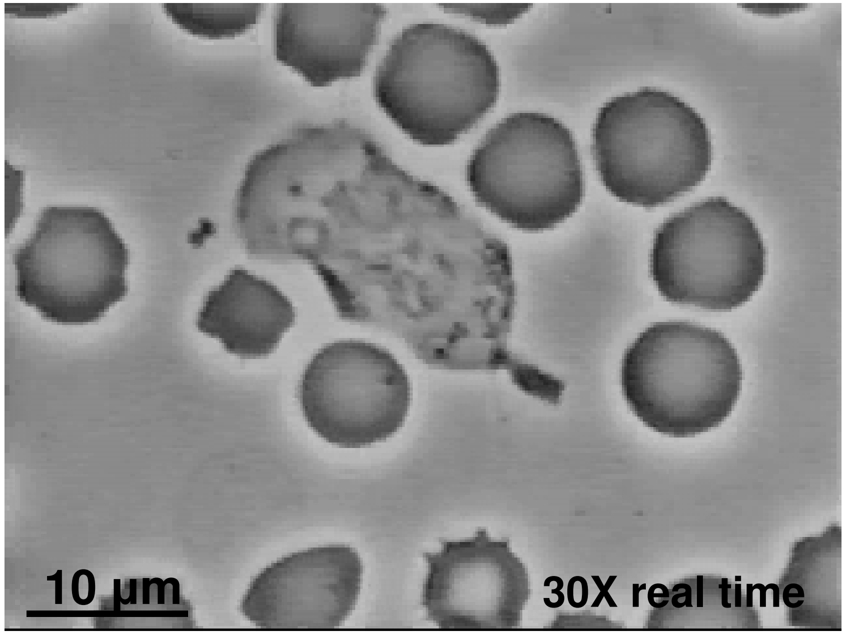
Cell movement is important for various biological phenomena:

- immune response (e.g. white blood cells)
- cancer metastasis
- wound healing



Monolayer of fibroblasts
("wound healing" on a coverslip)

Movie of monolayer of fibroblasts on a coverslip from Sheryl P. Denker and Diane L. Barber *JCB*, **159(6)**, 1087-1096 (2002)



a white blood cell chasing a bacterium

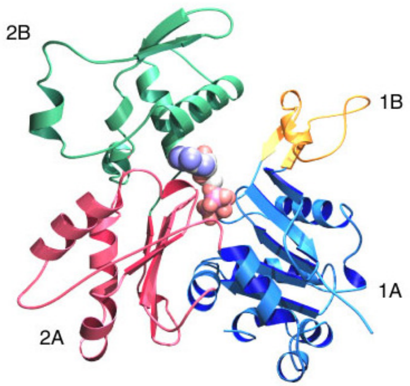
Movie by David Rogers, Vanderbilt University (taken in the 1950s)

Main player in cell crawling: **actin**

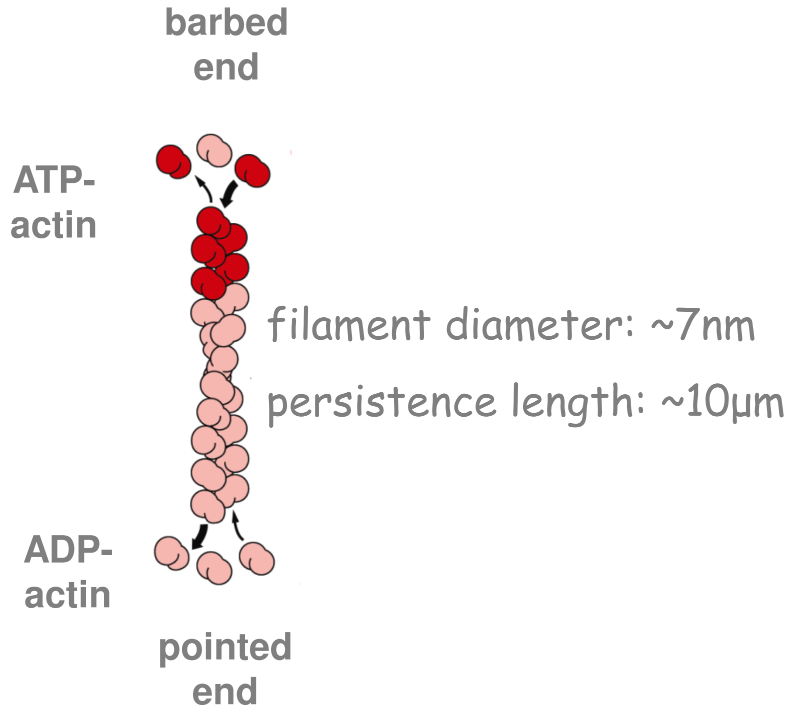
The majority of animal cells move by actin based motility

Actin is a globular protein; forms polarized helical filaments; hydrolyzes ATP

actin monomer ~5nm
(g-actin)

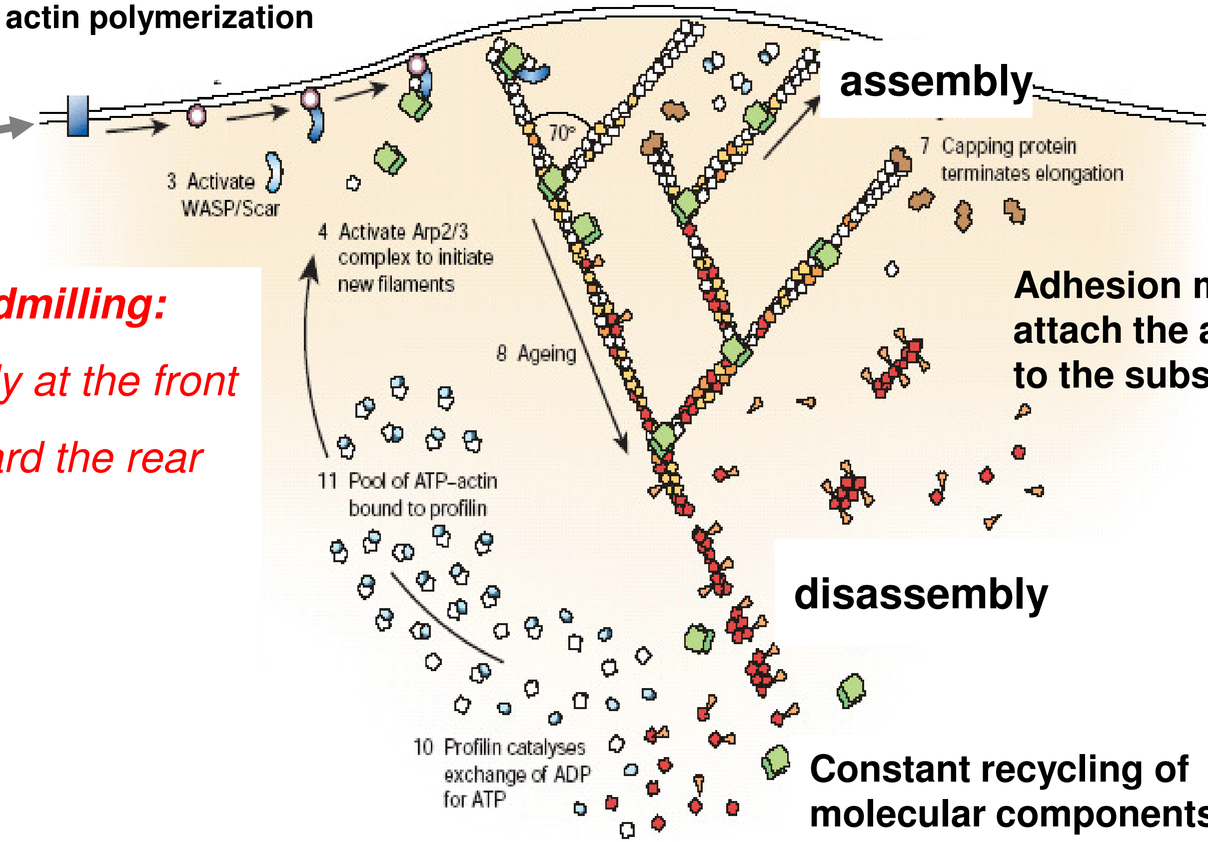


filamentous actin
(f-actin)



Biochemical model of the polymerization motor

Molecular players involved largely known; constant recycling of molecular building blocks.
Motor ultimately powered by ATP hydrolysis that ensures a supply of actin monomers.



Actin network treadmilling:

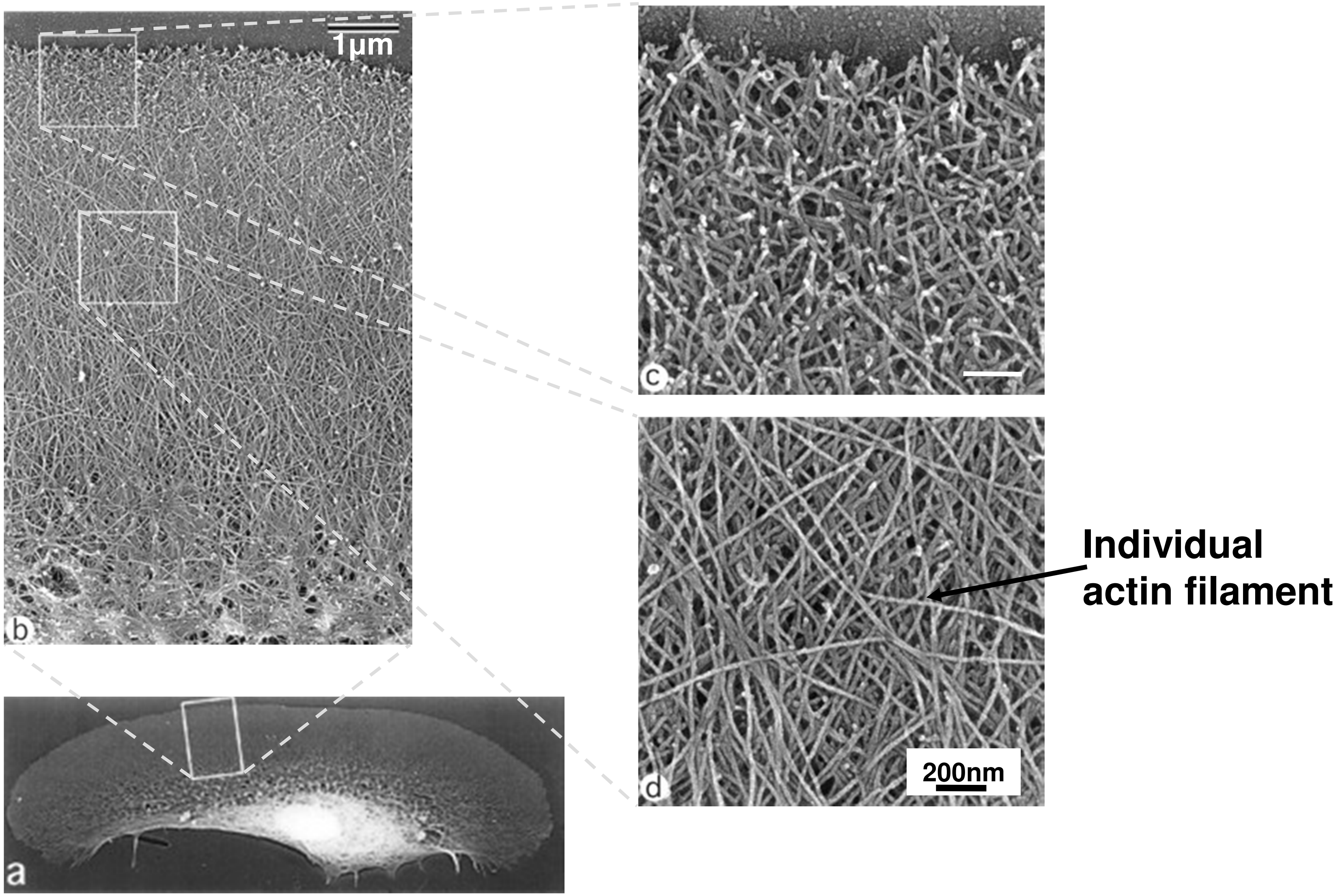
-assembly primarily at the front

-disassembly toward the rear

T. Pollard, *Nature* **422,** 741-745 (2003)

A zoom on the polymerization motor

An extended motor: dense cross linked actin meshwork (~10^{10} molecules)



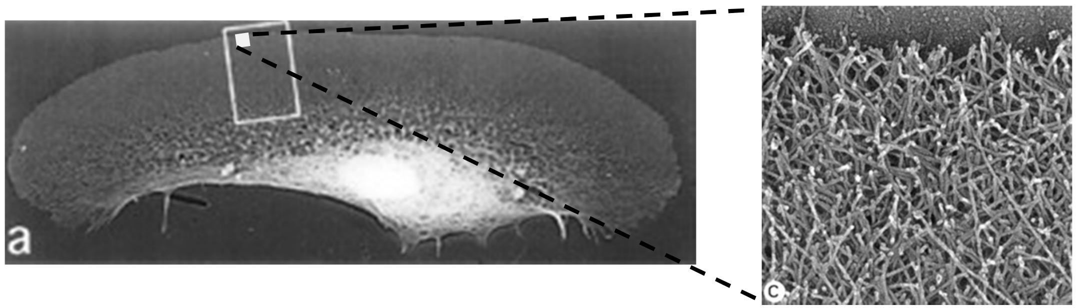
A crawling fish skin cell (keratocyte)

Svitkina TM *et al.* JCB. **139** 397-415 (1997)

The actin polymerization motor: a paradigm of *self organization*

- actin concentration ~ **10^{10} molecules/cell** (500μM)
- actin assembly rate at leading edge ~ **10^{6} molecules/sec**
- lifetime of actin monomer in meshwork ~ **30 s**
- total length of actin filaments ~ **10 cm filaments/cell**
- cell size ~ **10 μm**
- cell speed ~ 0.3 μm/s ~ **cell diameter/ 1 minute**

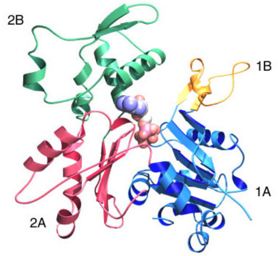
A cell moves by rebuilding its entire actin network every ~minute



Data adapted from: Abrahams *et al*., *Biophs. J.* **77** 1721-1732 (1999)

“Equivalent” problem…

Put the world population in an area the size of Budapest and *hope* they self-organize to move together at ~600kph



Individual molecule
Size: ~1nm

Thickness of human hair

Molecules/cell ~10^{10}
Length: ~10μm
Area: ~100μm^2
Speed: ~cell diameter/min
Filament length: ~10cm

Individual human being
Size: ~1m

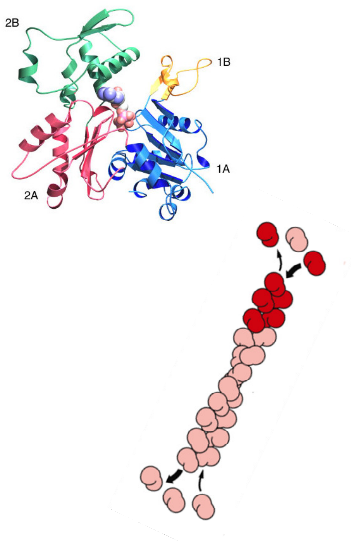
World population ~10^{10}
Length: ~10km
Area: ~100km^2
Speed: ~10km/min~600kph
Filament length: ~distance to moon

Self organization: from molecules to a moving cell

molecular building blocks → cellular structure and function

length~nm; time~s

length~10μm; time~hours



actin molecules

→ Biochemistry & Biophysics →

10μm 30X real time

Keratocyte: a fish skin cell

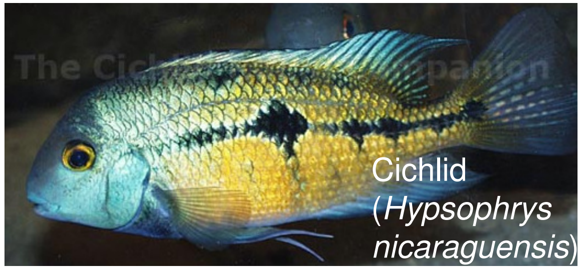
Self organization: from molecules to a moving cell

- Can we relate local dynamics at the molecular level to behavior at the cellular level?
- How are global shape and speed determined?
- What role does the surrounding membrane play?
- Can we come up with a mathematical model that quantitatively relates molecular parameters to global cell behavior?

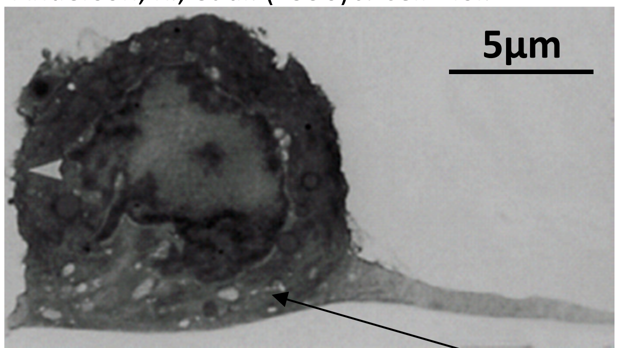
Complex problem….

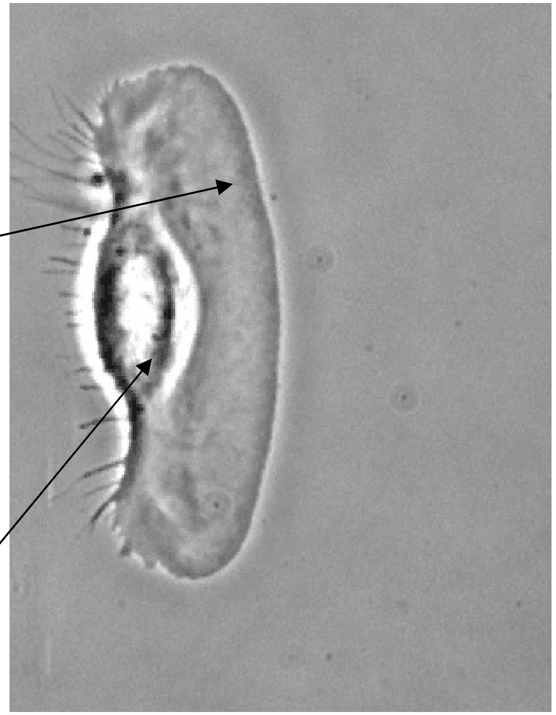
let's look for the simplest available model system

Model system: fish epithelial keratocytes



Anderson, K., et al. (1996) *J. Cell Biol.*



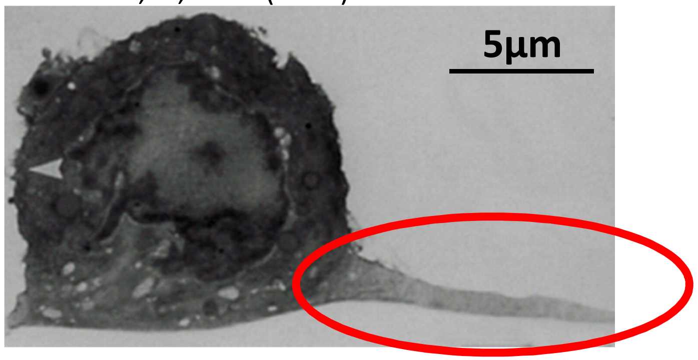


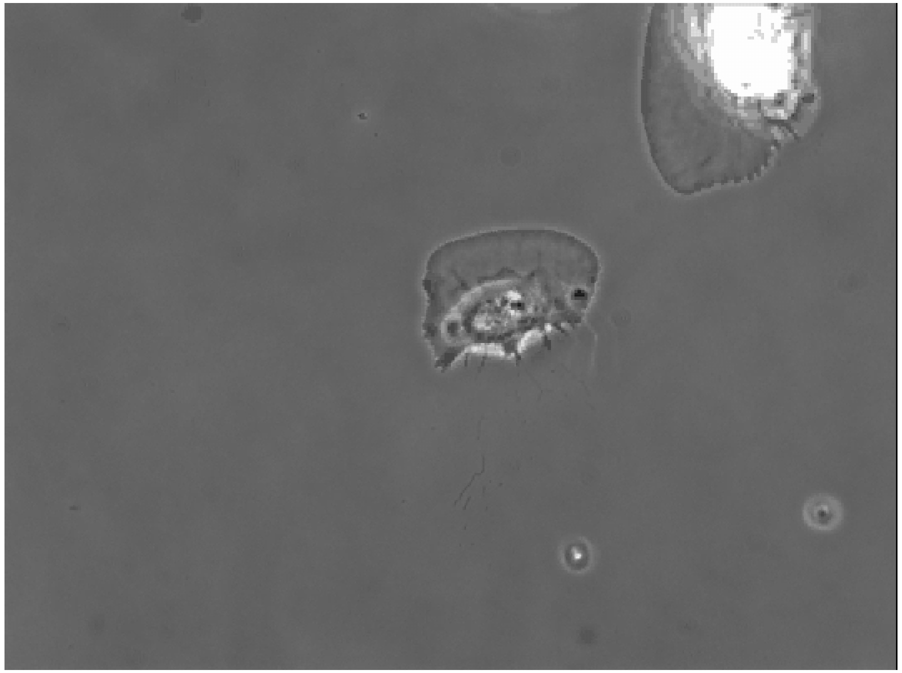
Advantages of keratocytes:

- **Persistent motion**- nearly constant shape, steady state motility
- **Fast moving**- up to 1 µm/s; fast turnover at the molecular level.
- **Flat lamellipodium**- 2D molecular machine (ideal for microscopy and modeling)

The lamellipodium is the motility apparatus

Anderson, K., et al. (1996) *J. Cell Biol.*





30µm

45X real time

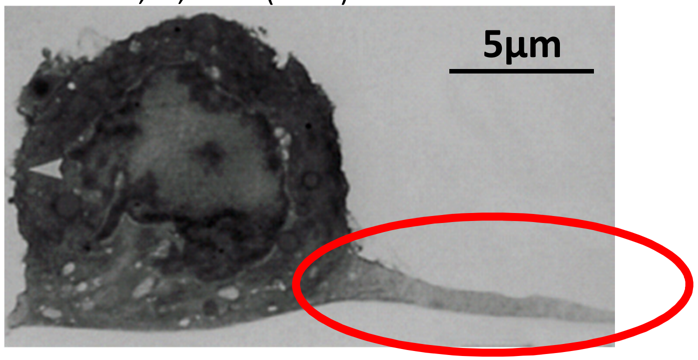
Cells can generate fragments which move on their own

Movie: Shlomit Yehudai-Reshef

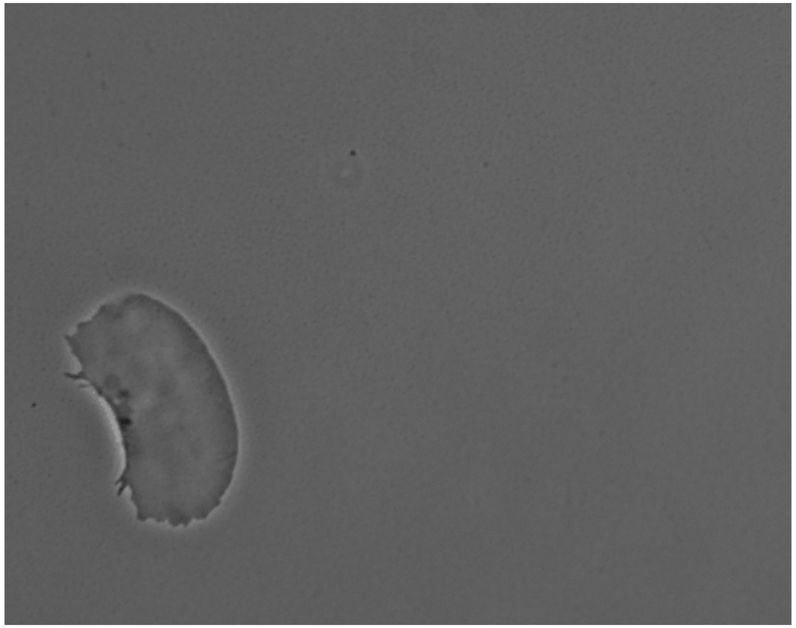
Lamellipodial fragments as a model system

- Essentially a stand alone lamellipodium; no cell body.
- Speed and persistence similar to cells.
- Keeps going for hours.

Anderson, K., et al. (1996) *J. Cell Biol.*



→*Simplest natural system to study lamellipodial motility*



10µm

30X real time

Movie: Noa Ofer

First paper on keratocyte fragments:
Euteneuer U, Schliwa M, *Ann N Y Acad Sci* **466**: 867-886 (1986)

Outline:

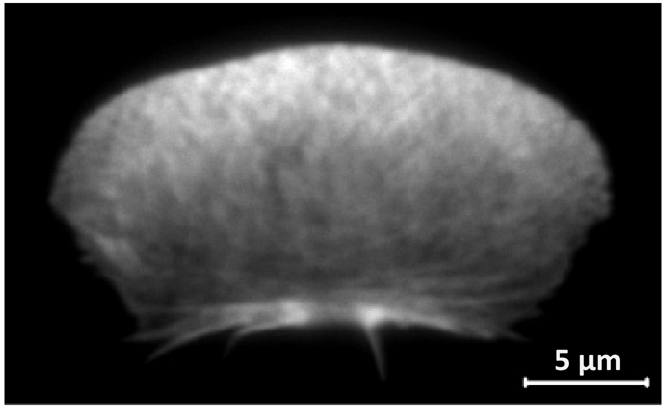
Part 1 - ***What determines shape and speed of fragments?***

- Characterization of the dynamics of keratocyte fragments
- Theoretical model: treadmilling actin network in a membrane bag

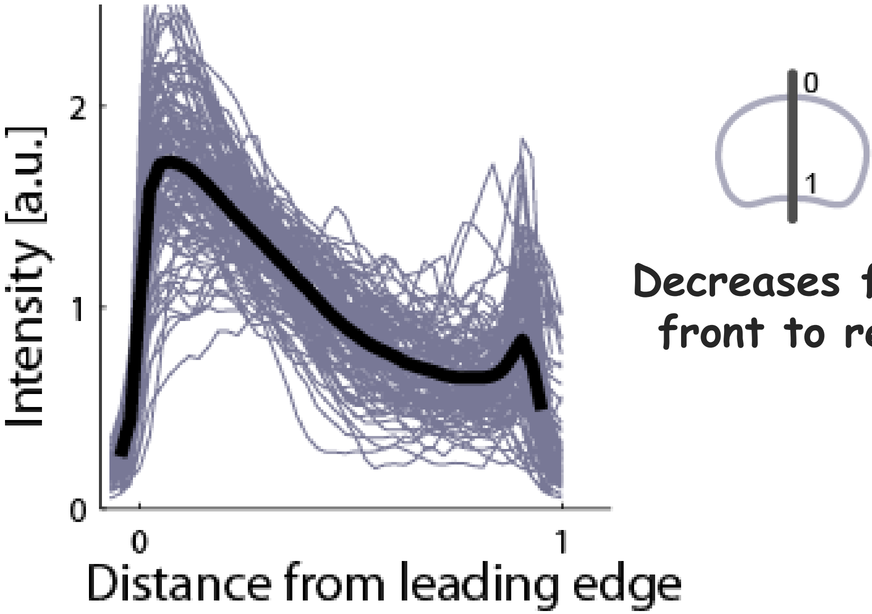
Part 2 - ***What determines membrane tension in motile cells?***

- Measurements of membrane tension in motile keratocytes
- Perturbations of the motility machinery and their effect on membrane tension
- Rapid increase in membrane area by fusion with giant vesicles

Actin network distribution in fragments

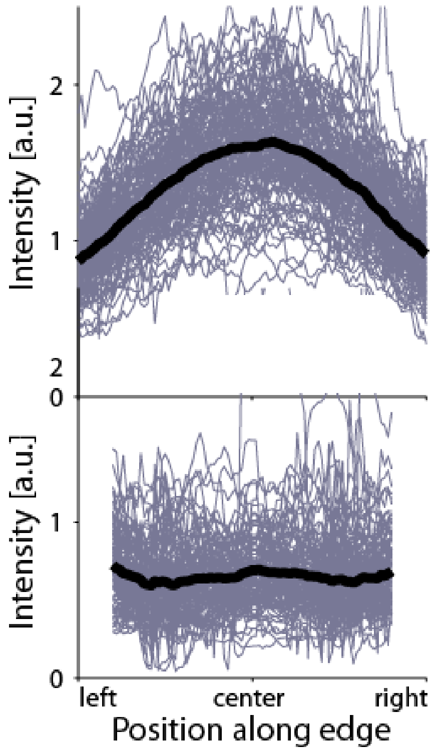


A fluorescent image of a fragment
fixed and stained with phalloidin



Decreases from front to rear

Peaked along leading edge



Flat along the rear

Data from a population of N=115 fragments

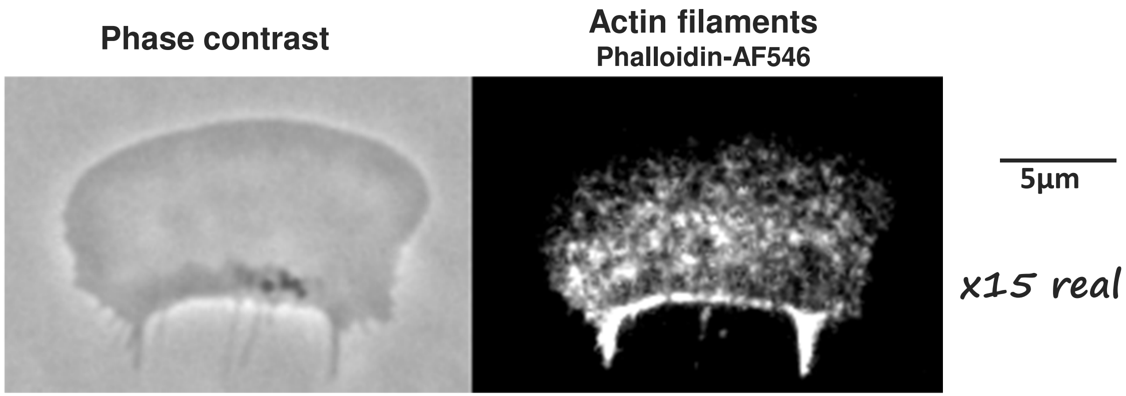
Black line- population average

Grey lines- individual fragments

Actin network dynamics in keratocyte fragments

Actin network flow visualized by Fluorescent Speckle Microscopy

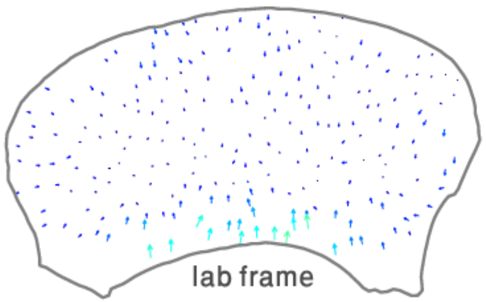
Cell frame
of reference



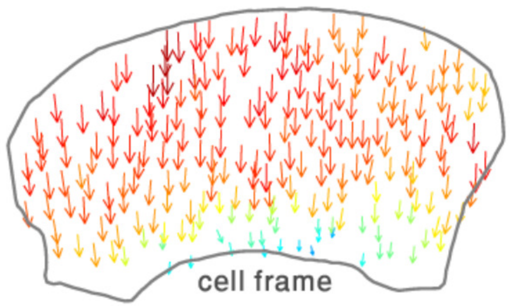
x15 real time

Actin network flow maps

V_{cell}=0.22μm/s



Actin network is
stationary in lab frame



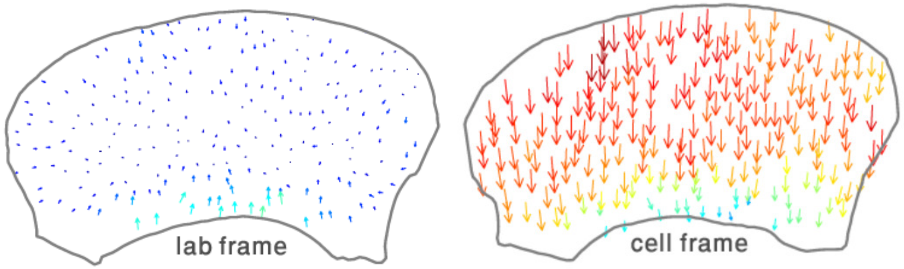
Actin network flow is moving
rearward in cell frame

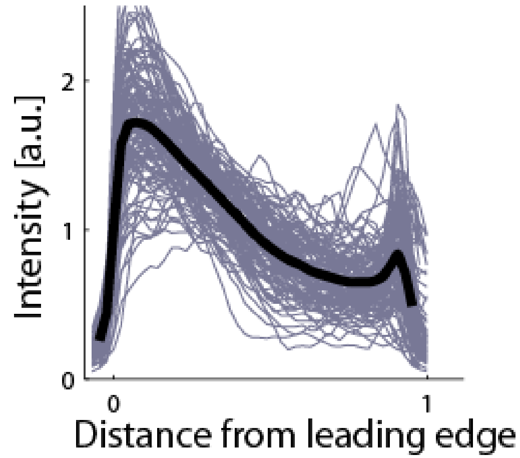
Noa Ofer

Actin network exhibits net disassembly from front to rear

- Actin density decays ~exponentially from front to rear

- Actin network is stationary in lab frame





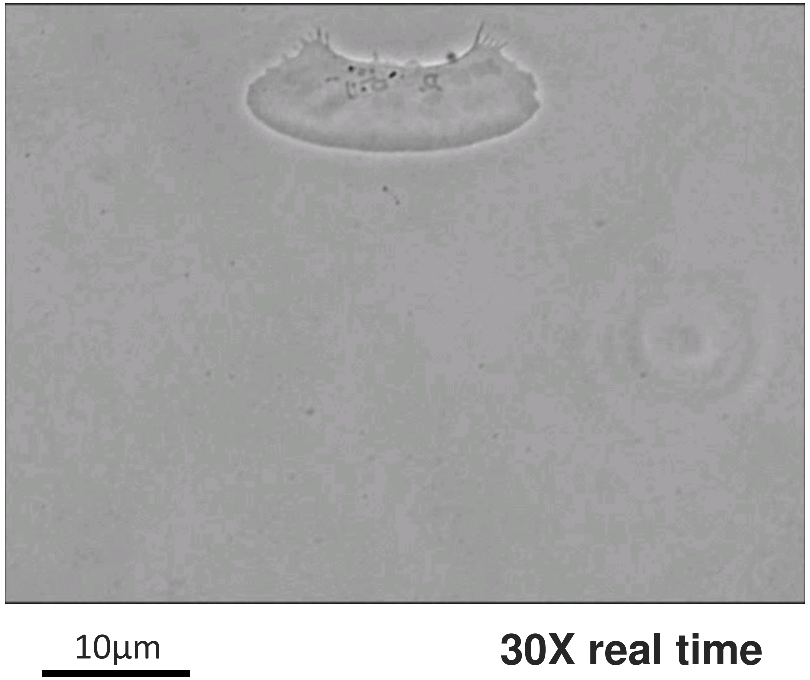
$$\frac{\partial B}{\partial t} = -V\frac{\partial B}{\partial s} - \frac{B}{\tau}$$

$$B(s) = B_C \exp(-s/V\tau)$$

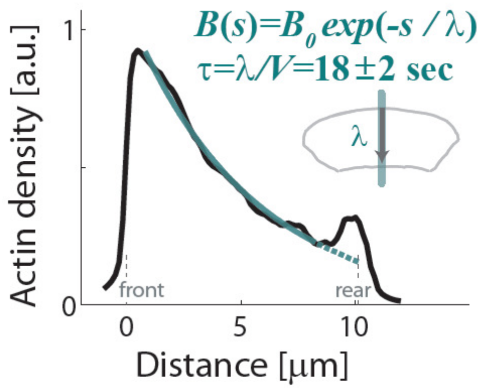
→ Constant actin network disassembly rate

Measurements of the actin disassembly time

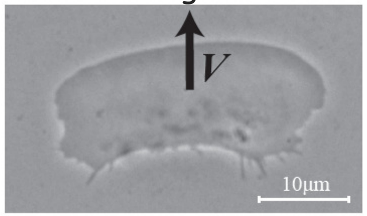
Combine time lapse imaging followed by fixation and staining within individual fragments

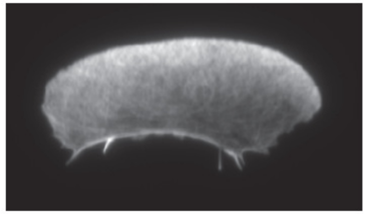


Movie: Noa Ofer



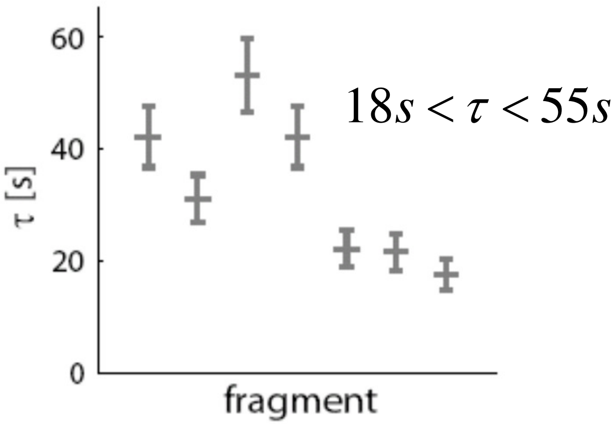
Measure speed in live fragments





Measure decay length of actin network density

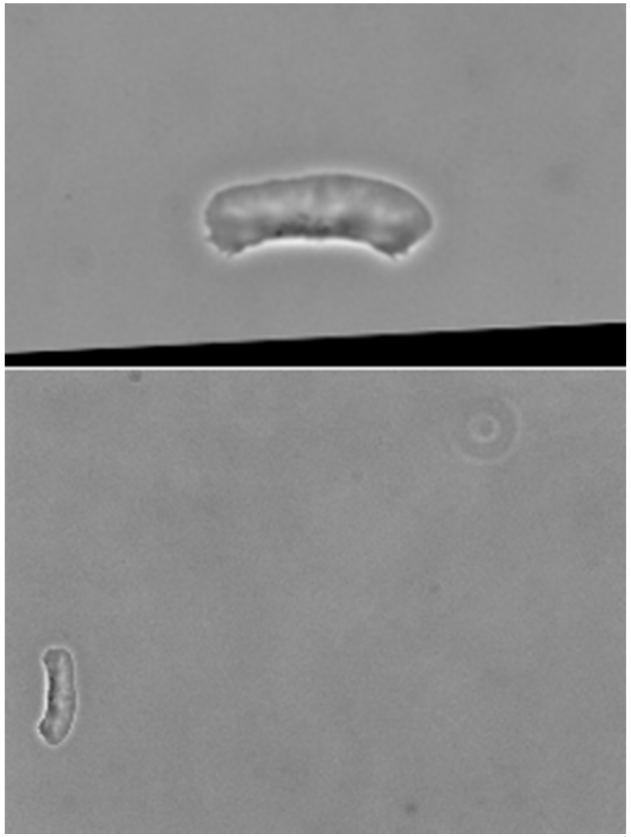
Disassembly in individual fragments



Following individual fragments over time

Time lapse movie of a fragment

Cell frame



Lab frame

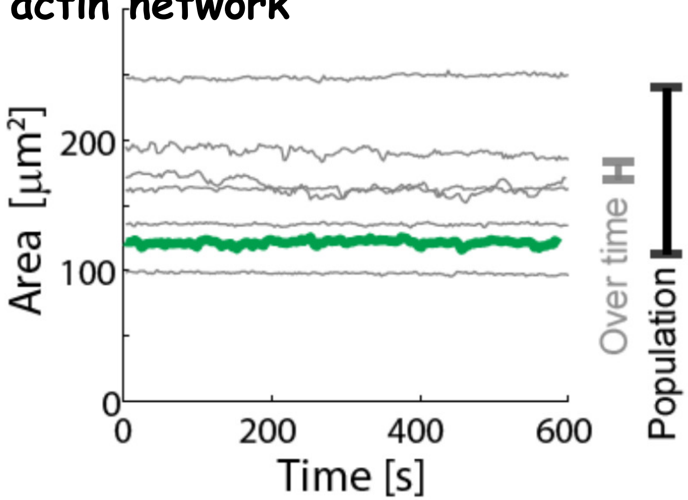
30µm

24X real time

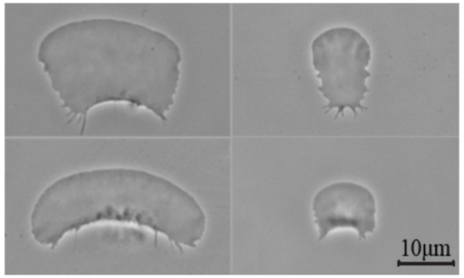
Movie: Noa Ofer

Area remains constant

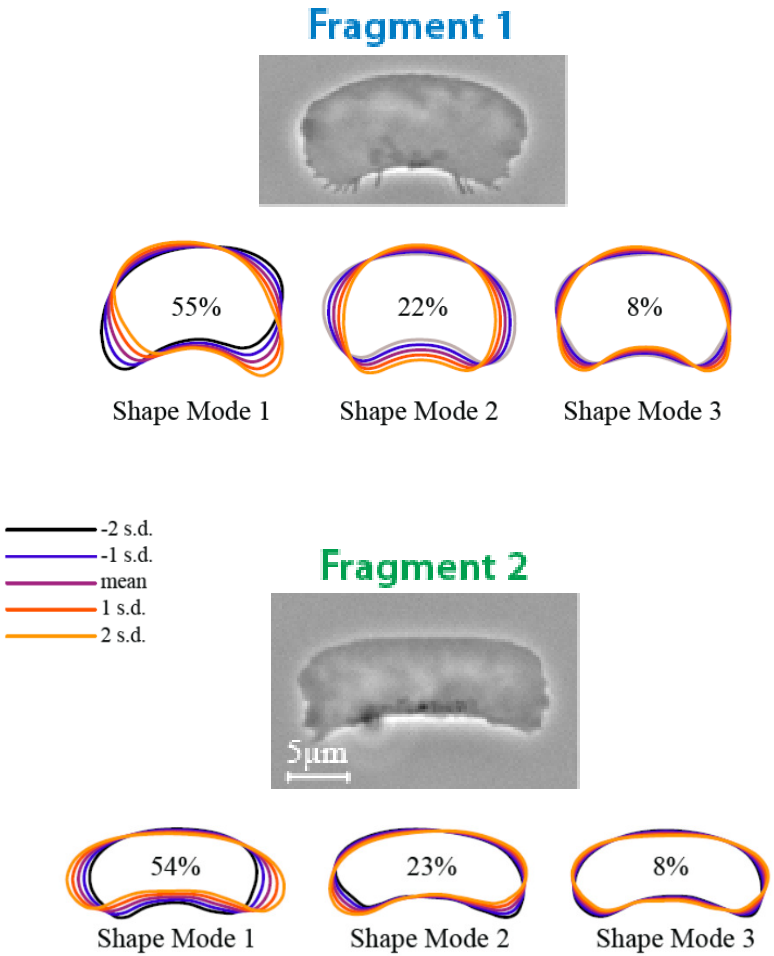
→ Plasma membrane area is fixed.
The membrane is stretched around actin network

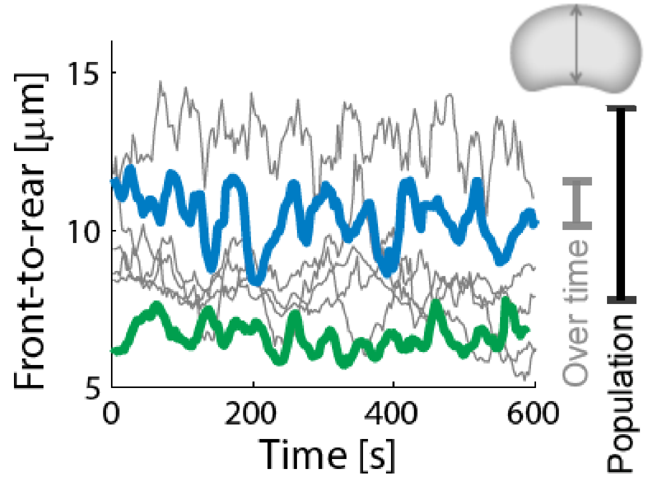


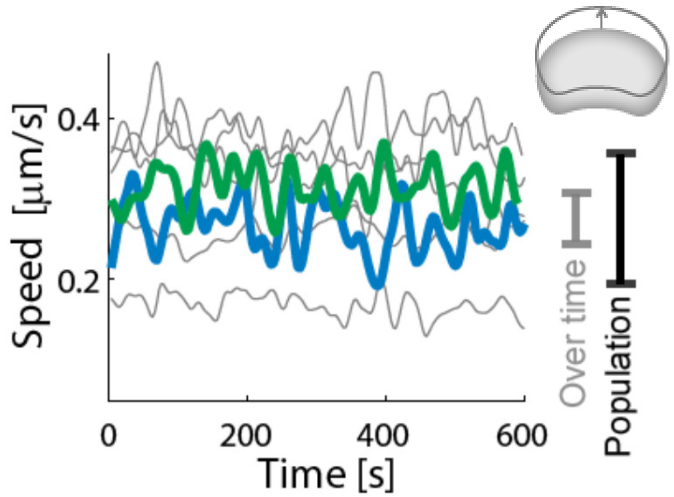
Fragment area varies between fragments



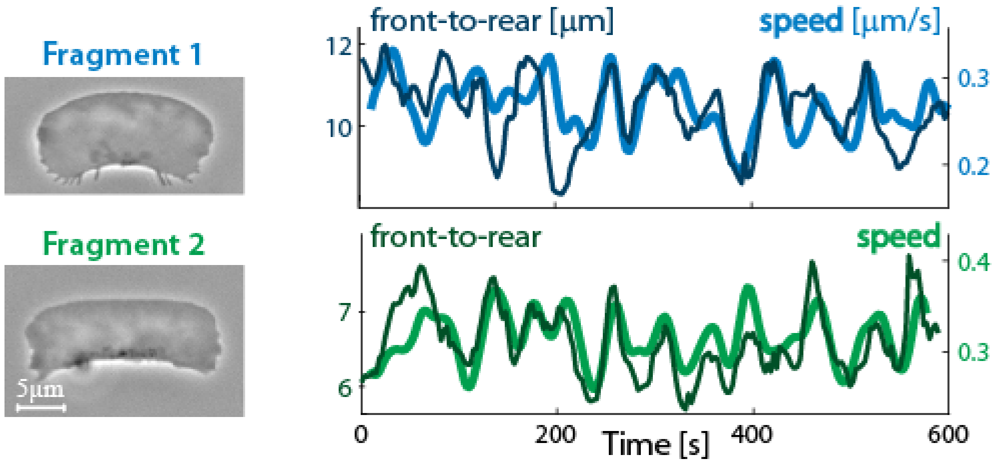
Shape and speed vary over time



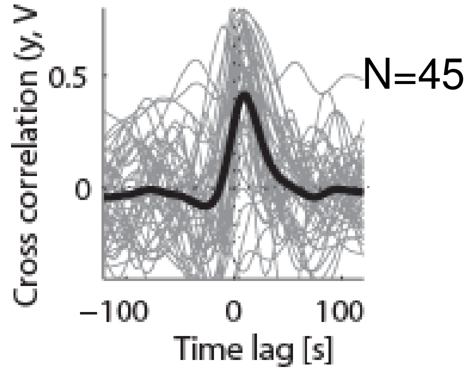


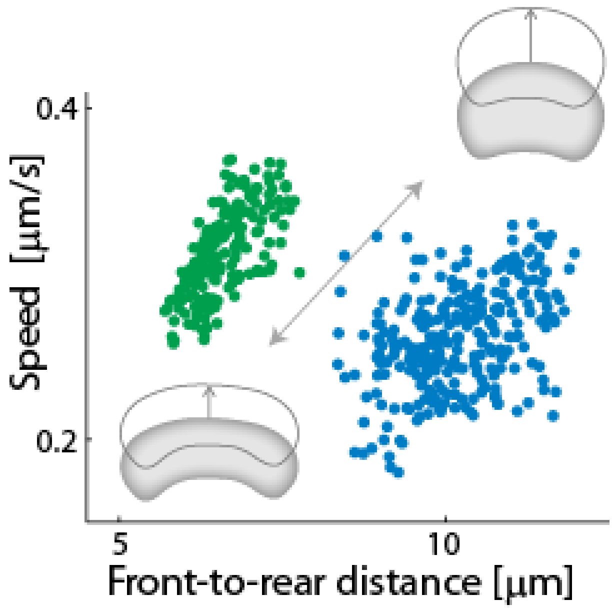


Shape and speed vary in a correlated manner



Cross correlation
front-to-rear and speed



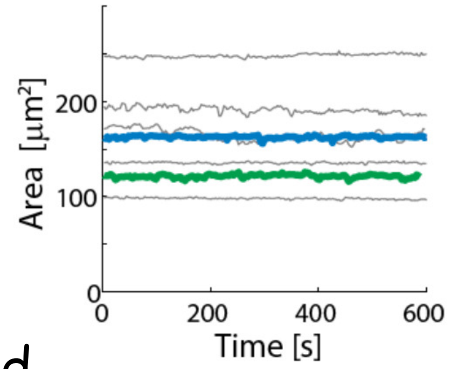


faster movement → larger front-to-rear distance

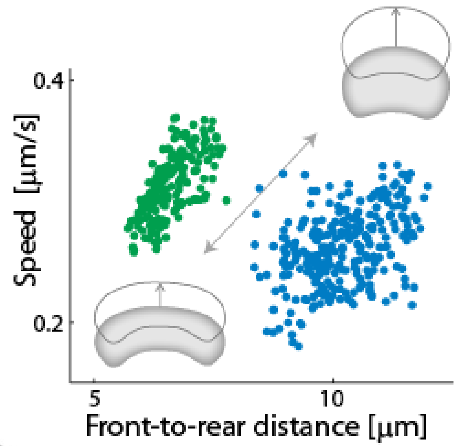
slower movement → smaller front-to-rear distance

Key experimental observations:

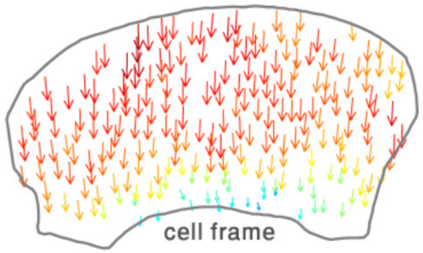
- Area remains constant

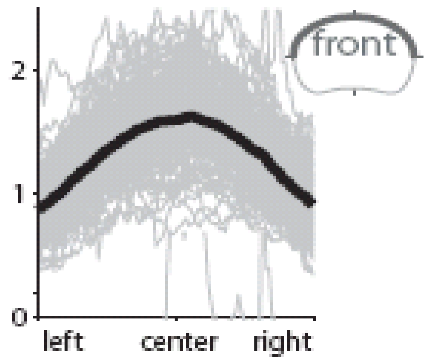


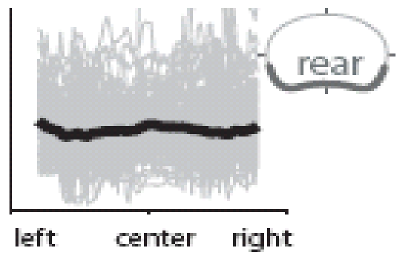
- Shape and speed are correlated



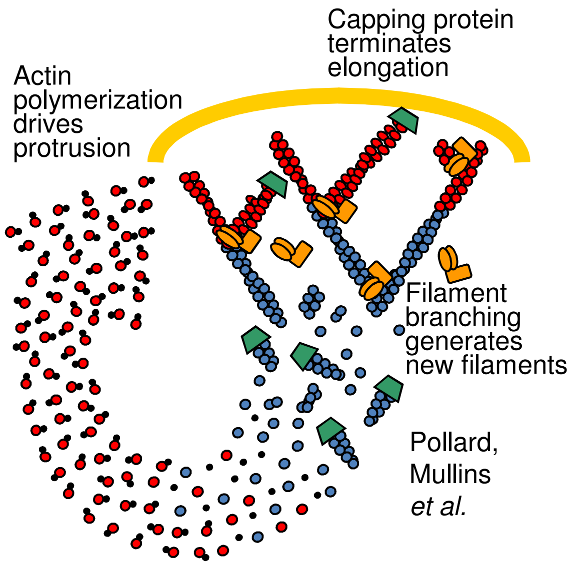
- Actin network treadmilling
  - -Constant actin flow rearward
  - -Constant disassembly rate
  - -Graded density along leading edge
  - -Flat distribution along rear





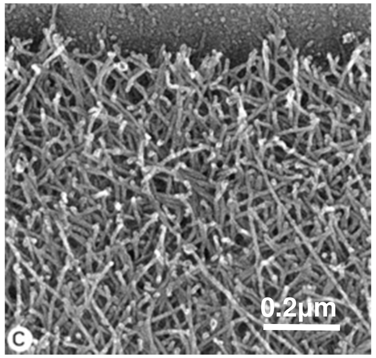


Can we relate the underlying molecular processes to global shape and speed?



~10^{10} molecules
size ~nm

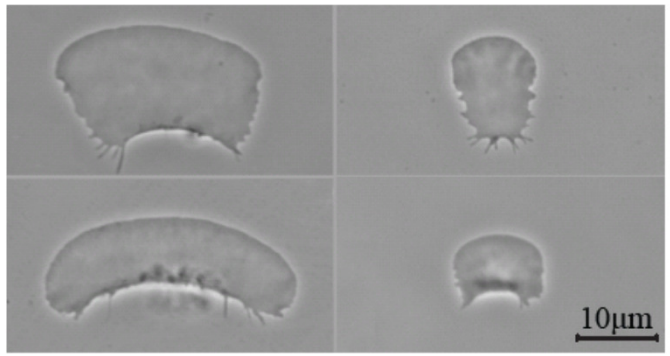
Svitkina, Borisy *et al.*



The actin network near the leading edge of a moving cell

?

modeling...



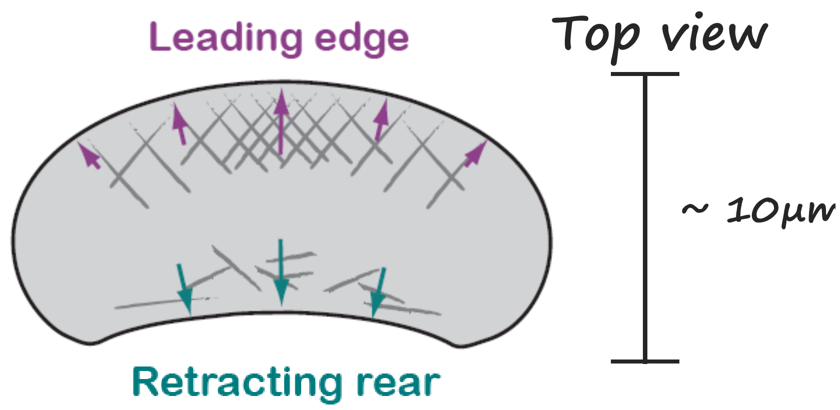
Lamellipodial fragments
size ~10µm

Theoretical model:
Actin network treadmilling in a membrane bag

Actin network treadmilling
assembly at the front; disassembly toward the rear.

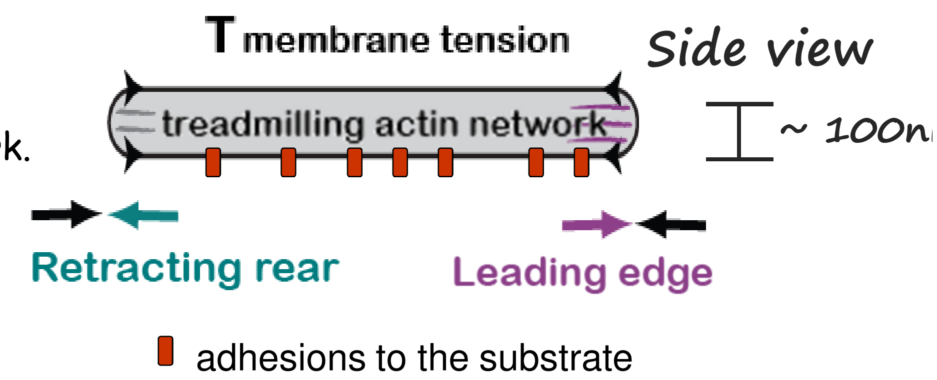
Inextensible membrane → constant area.

Membrane tension is generated by the motility machinery; pushing at the front; resisting retraction at the rear.



-*At the front* : membrane tension applies an opposing force on the polymerizing actin network.

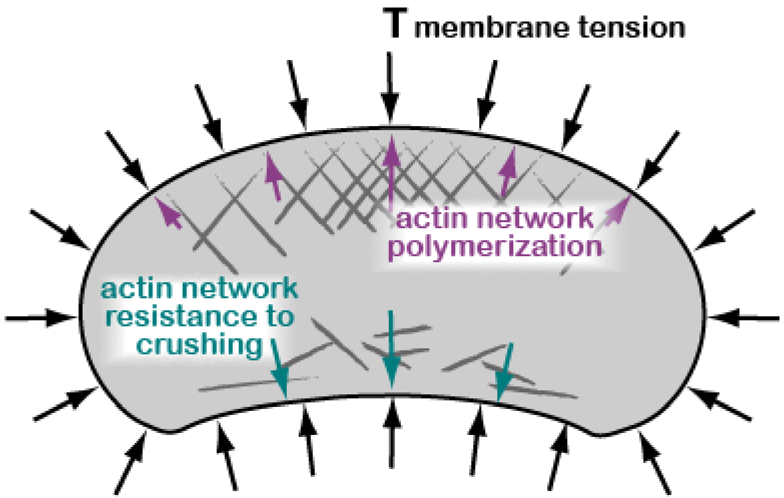
-*At the rear* : retraction is driven by forces due to membrane tension.



Membrane tension couples front and rear

Local force balance between actin network and membrane tension determines global shape.

Membrane tension is constant along the boundary.



Front: Actin meshwork polymerization force is balanced by membrane tension.

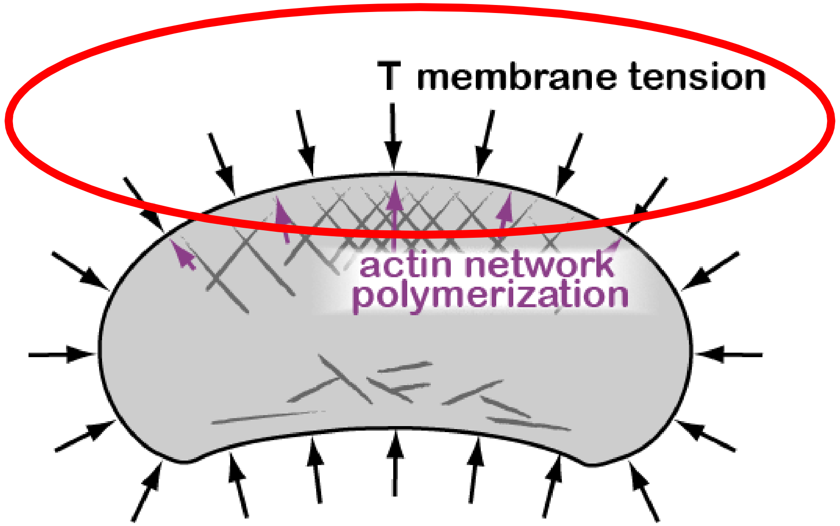
Rear: Actin meshwork resistance to crushing is balanced by membrane tension.

2D model of lamellipodial motility

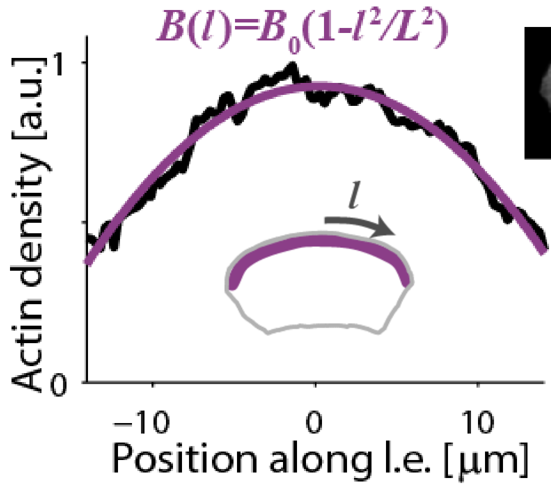
- Disassembly sets a 'clock' that determines front-to-rear distance.
- Membrane tension mechanically couples protrusion at the front and retraction at the rear.

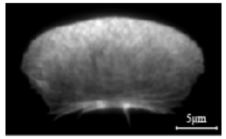
Force balance at the front

between membrane tension and actin network polymerization



Filament density along leading edge is graded





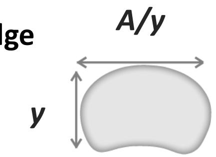
$$f(l) = \frac{T}{B(l)}$$

Constant Tension
force per unit length

Force per filament

Filament density is graded

B(l)	Actin density along the leading edge
B_o	Actin density at front centre
2L	Distance between rear corners



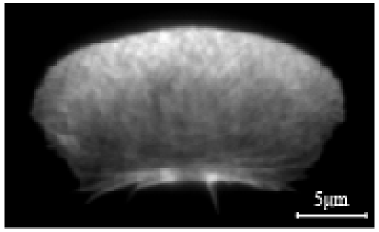
$$L = y + A/2y$$

KK, Z Pincus, G Allen, E Barnhart, G Marriott, A Mogilner, J Theriot, *Nature* (2008)

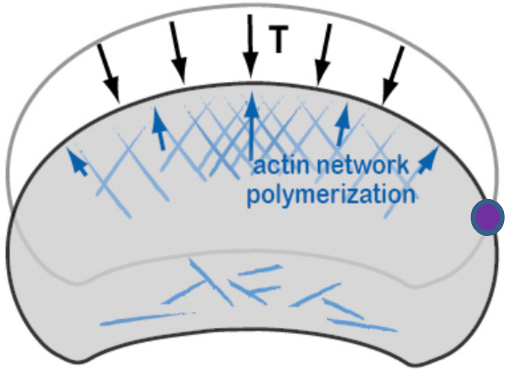
Protrusion is stalled at front corners

Force per filament increases toward the sides

$$f(l) = \frac{T}{B(l)}$$



Front corners are defined by where protrusion is stalled: $f = f_{stall}$



$$f = \frac{T}{B_{sides}} = f_{stall}$$

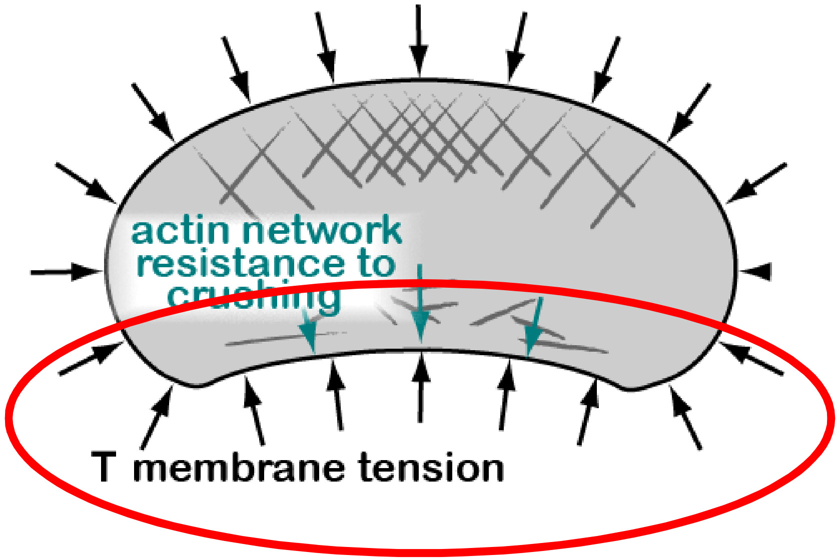
$$T = f_{stall} B_{sides}$$

$$1 - \left(\frac{1}{1 + {2y^2}/{A}} \right)^2 = \frac{T}{B_0 f_{stall}}$$

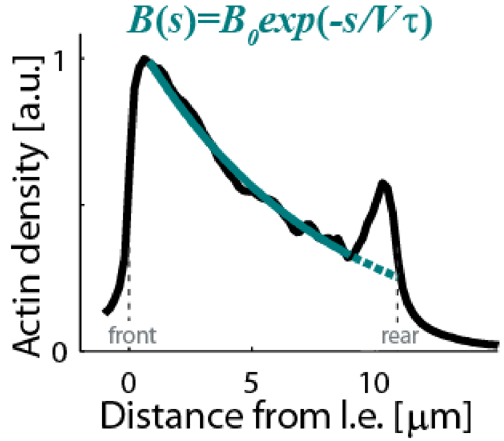
KK, Z Pincus, G Allen, E Barnhart, G Marriott, A Mogilner, J Theriot, *Nature* (2008)

Force balance at the rear

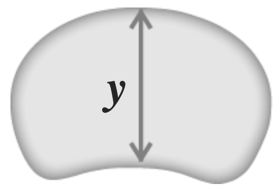
between membrane tension and actin network resistance



Rear boundary is defined by where actin network has disassembled sufficiently so the membrane tension can crush it

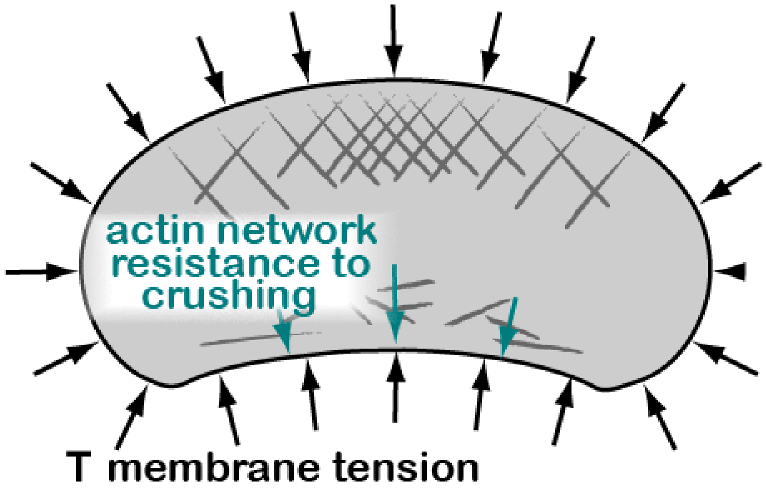


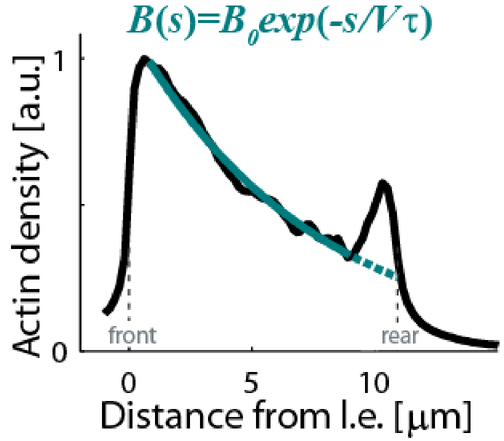
$$B_{rear} = B(y) = B_0 \exp(-y/V\tau)$$



Force balance at the rear

between membrane tension and actin network resistance





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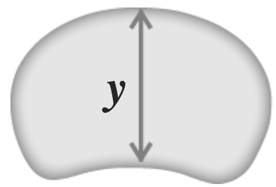


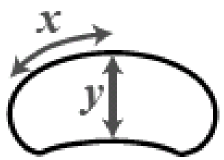
Force needed to crush network $\propto$ Actin network density

$$T = kB_{rear}$$

k – Breaking force per filament

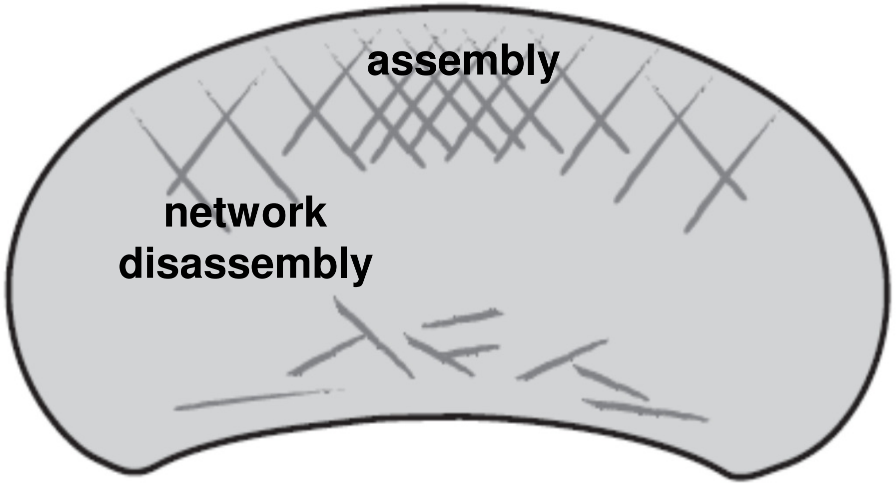
$$T = kB_0 \exp(-y/V\tau)$$

'Actin disassembly' clock model



front corners:

$T = f_{stall} B(x) = f_{stall} B_0 (1-(1+2y^2/A)^{-2})$



rear edge:

$T = kB(y) = kB_0 exp(-y/V\tau)$

Front and rear coupled by the membrane;
Membrane tension is the same everywhere

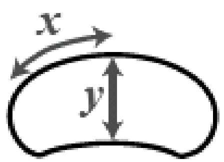
front-to-rear distance
determined by the time needed
for disassembly

$$y \sim V \tau$$

V - speed

τ – disassembly time

'Actin disassembly' clock model



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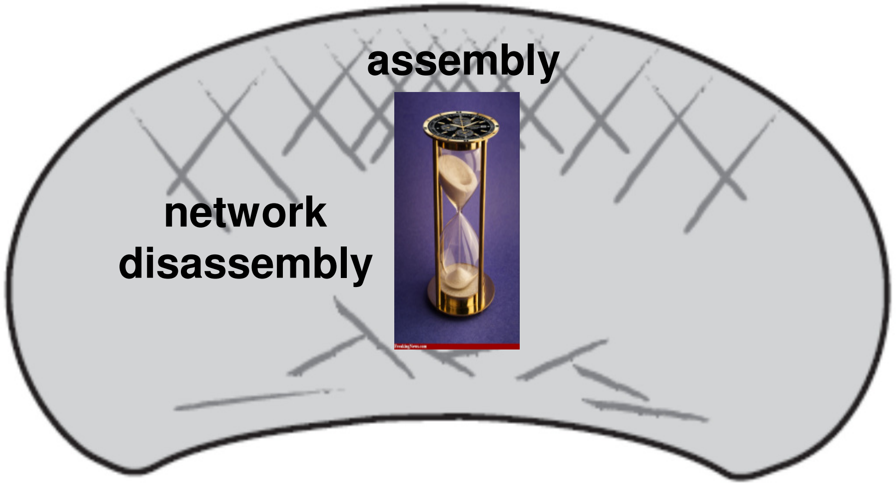


rear edge:

$T = kB(y) = kB_0 exp(-y/V\tau)$

Membrane tension is the same at the front and at the rear

$$\exp\left(-\frac{y}{V\tau}\right) = \varepsilon\left(1-\left(1+\frac{2y^2}{A}\right)^{-2}\right)$$

$$\varepsilon = \frac{f_{stall}}{k} < 1$$

Simple solution for $\varepsilon << 1$

$$V = \frac{-1}{\log\varepsilon}\frac{y}{\tau}$$

Model parameters:

A	area	v	cell speed	$\varepsilon = f_{stall}/k$
τ	disassembly time	f_{stall}	stall force (per filament)	
B_0	barbed end density	k	breaking force (per filament)	

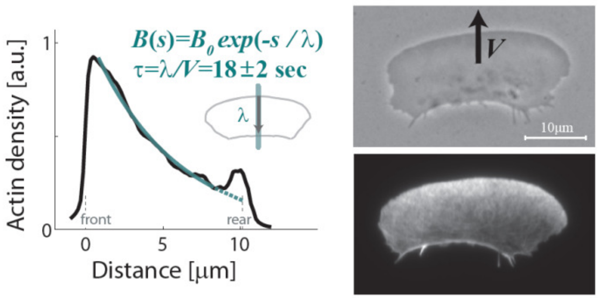
Model predicts observed correlation between shape and speed

Model prediction

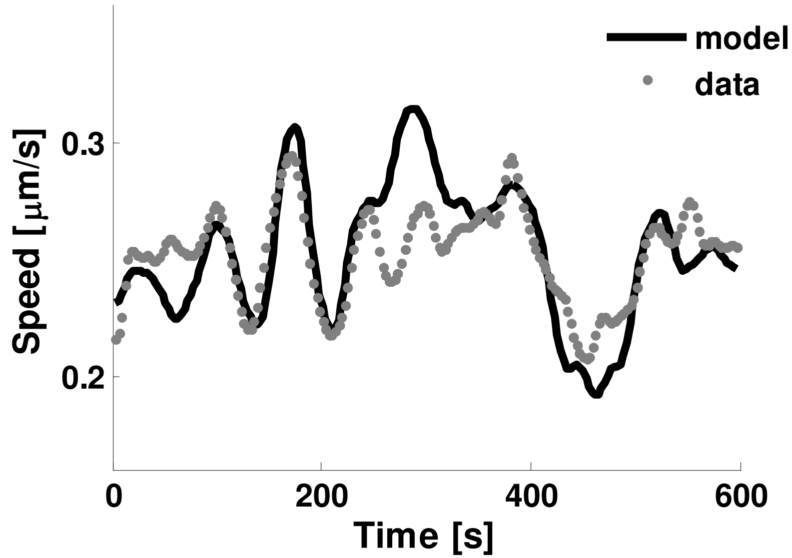
$$V = \frac{y}{\tau}\frac{-1}{\log\varepsilon + \log\left(1-\left(1+\frac{2y^2}{A}\right)^{-2}\right)}$$

Look at individual fragments

use measured A, τ; fit ε



Model prediction for time series of individual fragment



Model parameters:

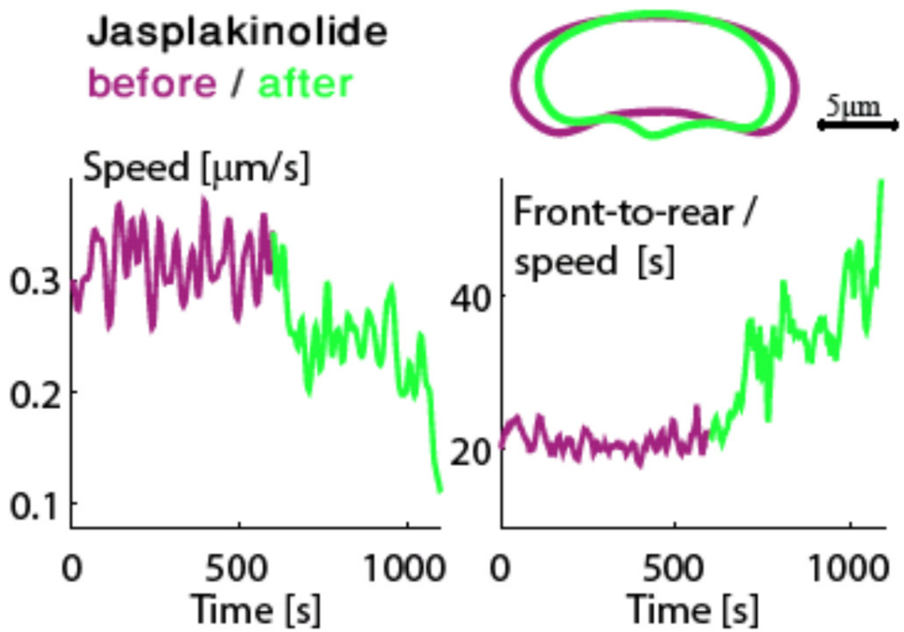
A	area	v	cell speed $\varepsilon = f_{stall}/k$
τ	disassembly time	f_{stall}	stall force (per filament)
B_0	barbed end density	k	breaking force (per filament)

Direct test of 'disassembly clock' model

What happens if we slow down actin disassembly?

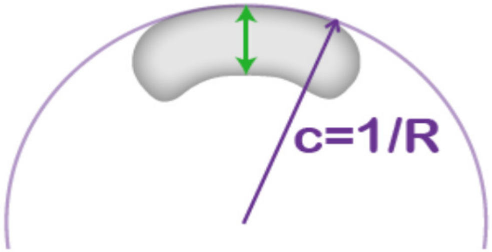
Biochemically slow down disassembly by adding jasplakinolide (stabilizes filaments and slows disassembly)

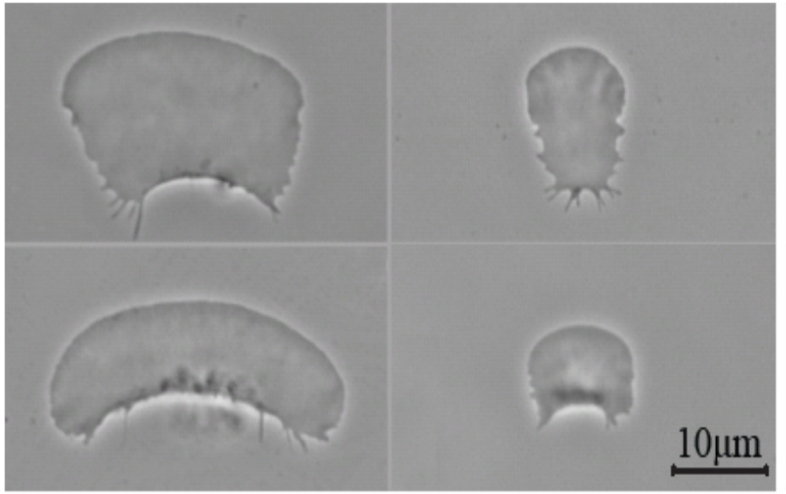
→ **expect** $\tau \sim y / V_{cell}$ **will increase**



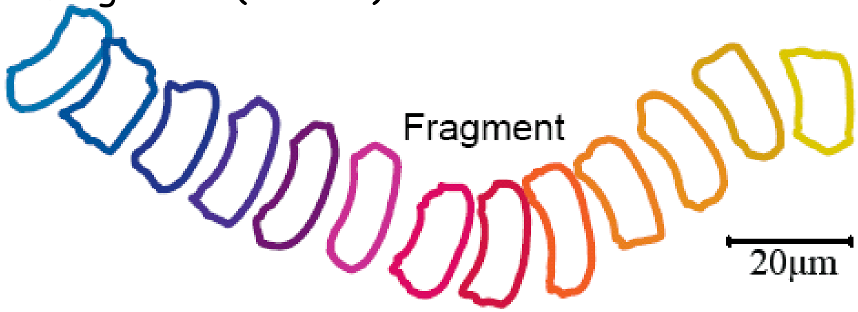
The model also predicts the shape of the leading edge

Curvature of the leading edge



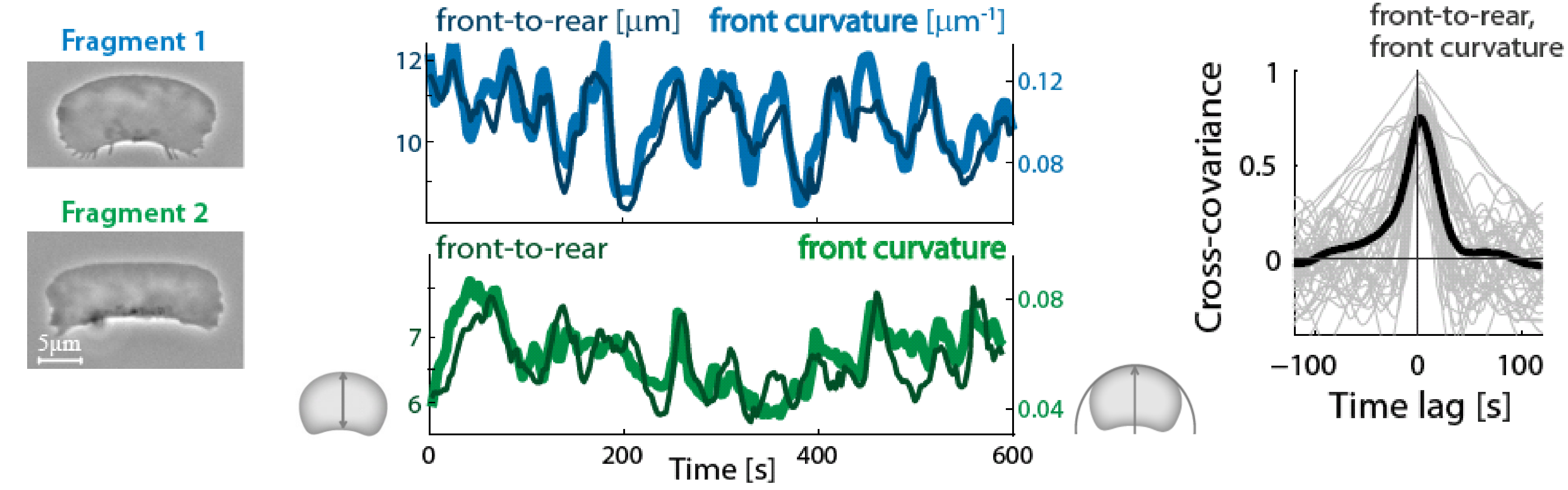


Contours of from a time lapse movie of a fragments (dt=48s)

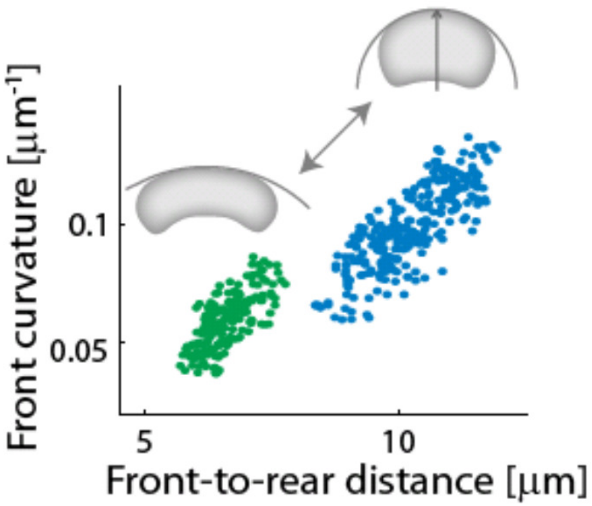


Leading edge curvature varies between fragments and over time

Front-to-rear distance is highly correlated with curvature of the leading edge



rounder shape → larger front-to-rear distance



flatter shape → smaller front-to-rear distance

Model predicts correlation between front-to-back distance and front curvature

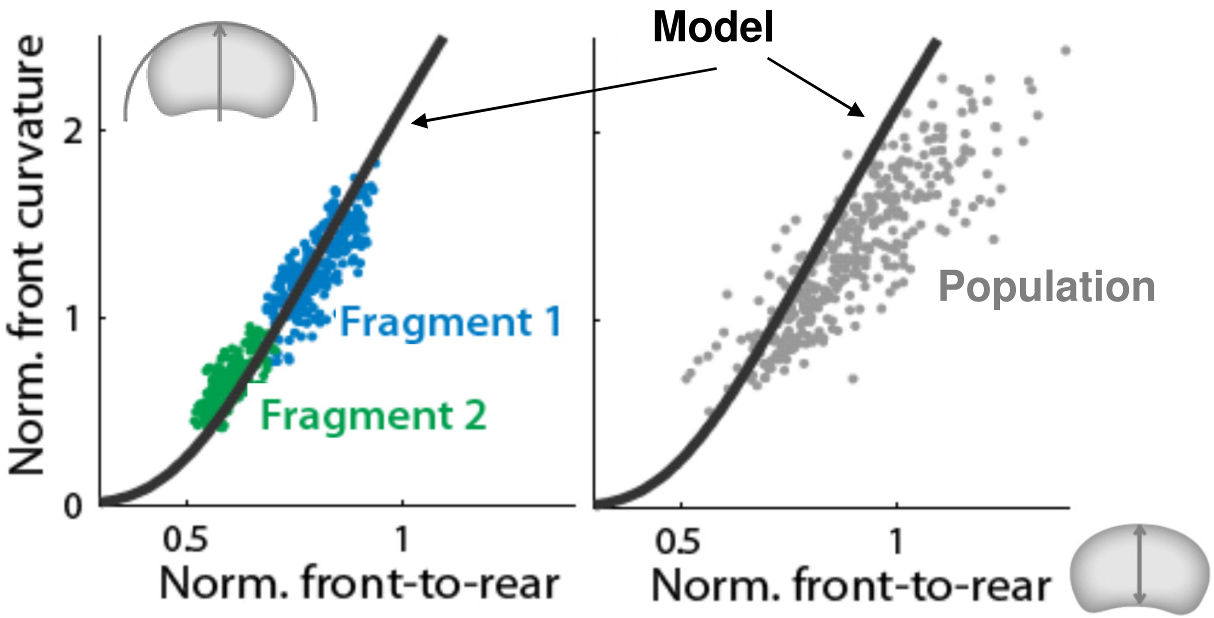
local filament density	force per filament	local protrusion rate

$$B(l) = B_0\left(1-\left(\frac{l}{L}\right)^2\right) \rightarrow f(l) = \frac{T}{B(l)} \rightarrow V(l) \rightarrow \text{shape}$$

$$R_{le} \approx \frac{l}{\theta} \approx \frac{L}{2}\sqrt{\frac{V/B_0}{2dV/dB}}$$

$\sqrt{A}$: length unit

$$\tilde{R}_{le} = \frac{R_{le}}{\sqrt{A}} \qquad \tilde{y} = \frac{y}{\sqrt{A}}$$



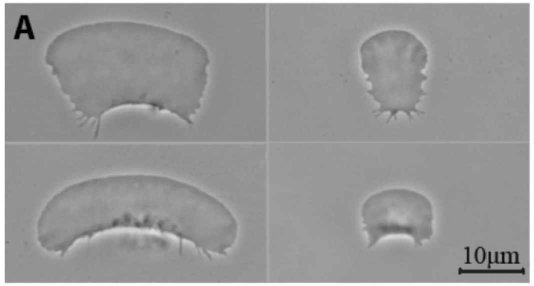
Summary I

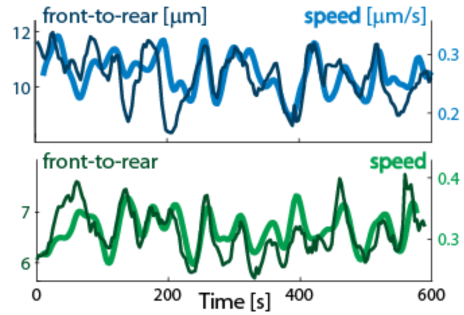
•Fragment area remains constant while front-to-rear distance and speed vary in a correlated manner.

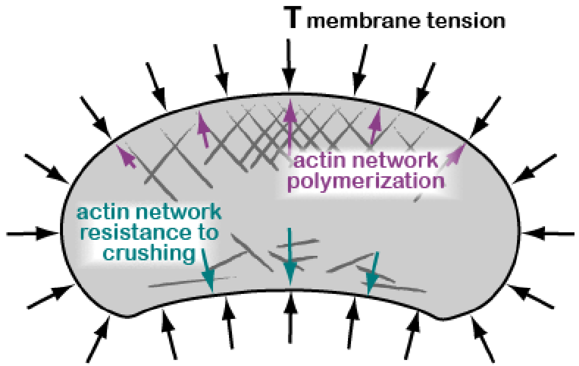
•Global shape and speed are determined by local force balances.

•Membrane tension arises from a dynamic interplay between the actin cytoskeleton and the cell membrane. Tension mechanically coordinates protrusion at the front with retraction at the rear.

•Model of actin network treadmill coupled to membrane tension explains observed shapes in a *quantitative* manner: force balance between actin polymerization and membrane tension along leading edge; "Disassembly clock" defines rear.

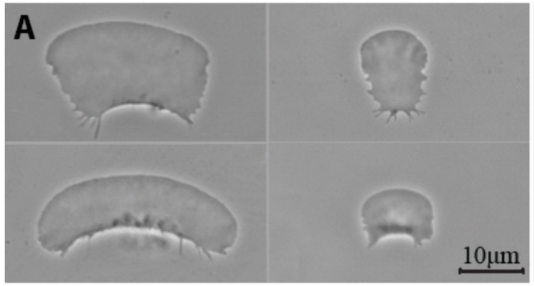




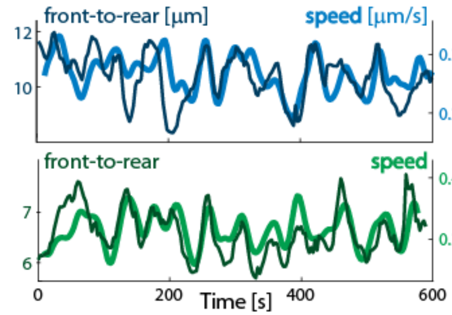


Summary I

•Fragments are the simplest natural model system for actin-based motility.

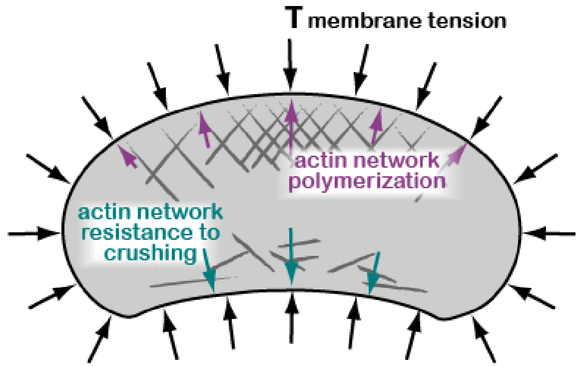


•Fragment area remains constant while front-to-rear distance and speed vary in a correlated manner.



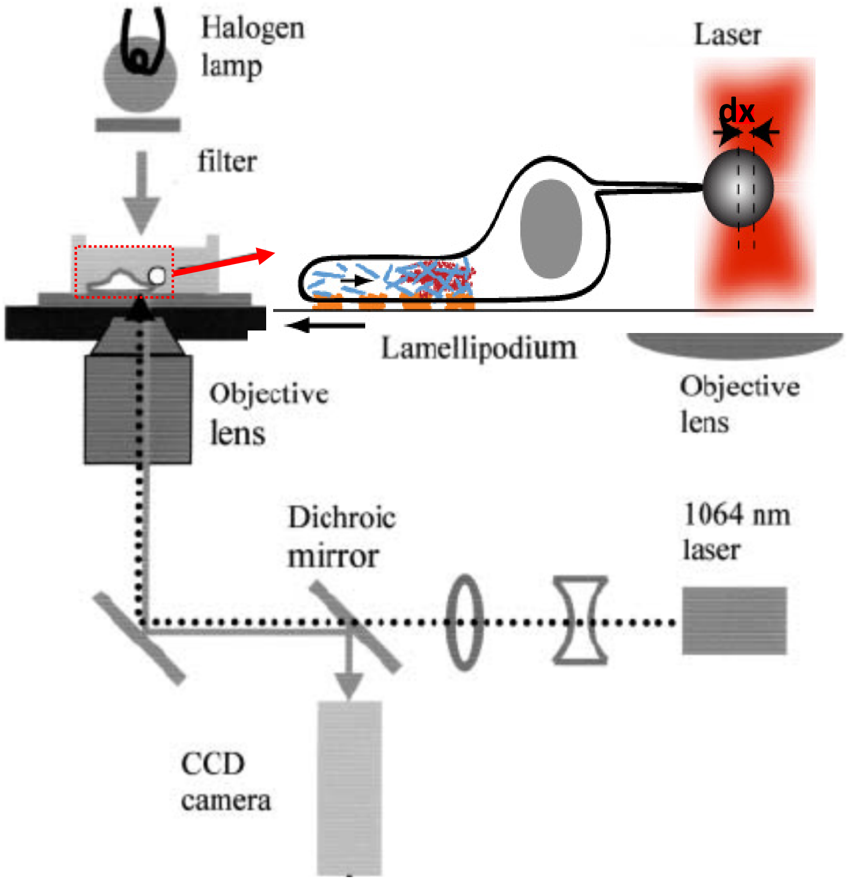
•***Membrane tension arises from a dynamic interplay between the actin cytoskeleton and the cell membrane. Tension mechanically coordinates protrusion at the front with retraction at the rear.***

•Global shape and speed are determined by local force balances.



•Model of actin network treadmill coupled to membrane tension explains observed shapes in a *quantitative* manner: force balance between actin polymerization and membrane tension along leading edge; "Disassembly clock" defines rear.

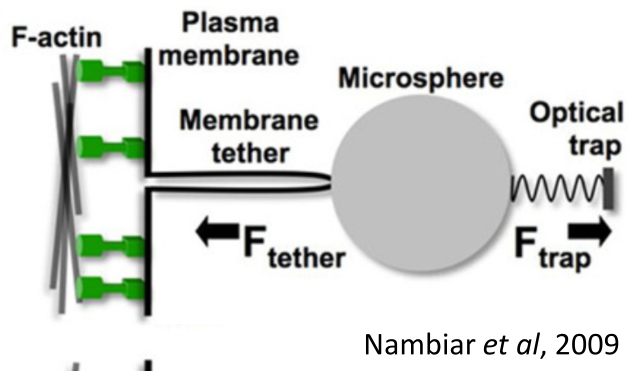
Membrane tension measurements in keratocytes



Hochmuth, Sheetz *et al.* (1996)

Dai and Sheetz (1998)

Membrane tension can be measured by pulling a membrane tether



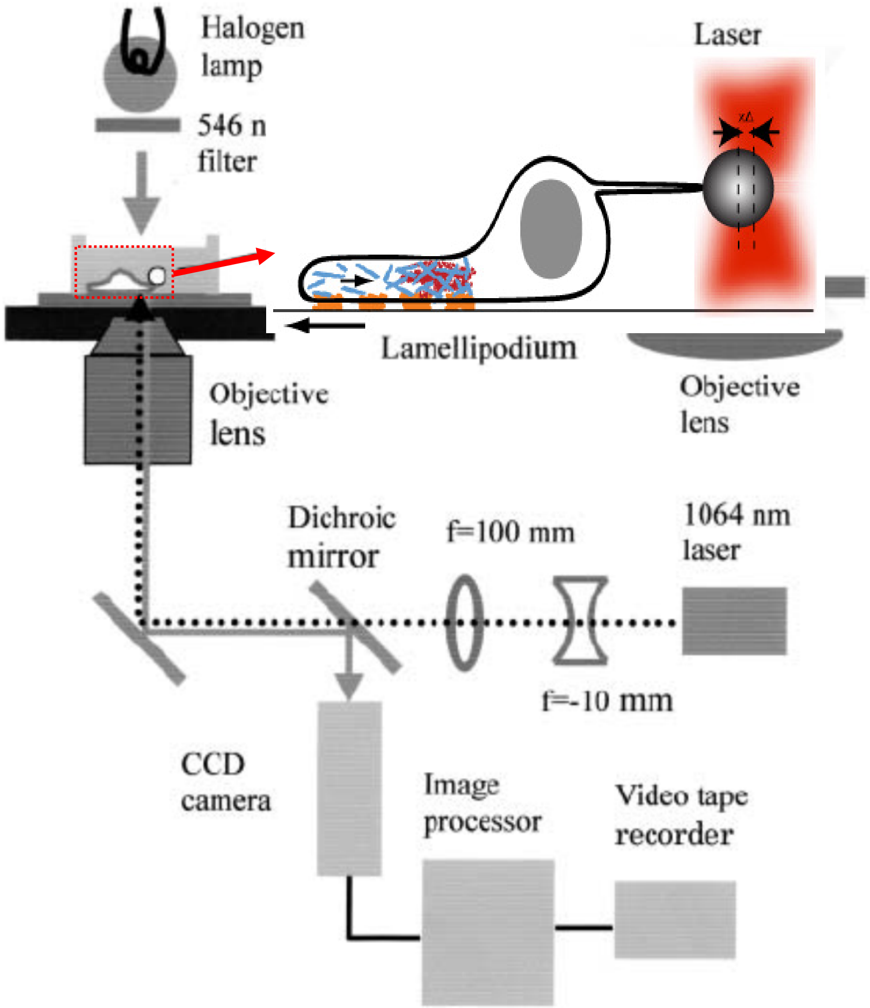
Nambiar *et al*, 2009

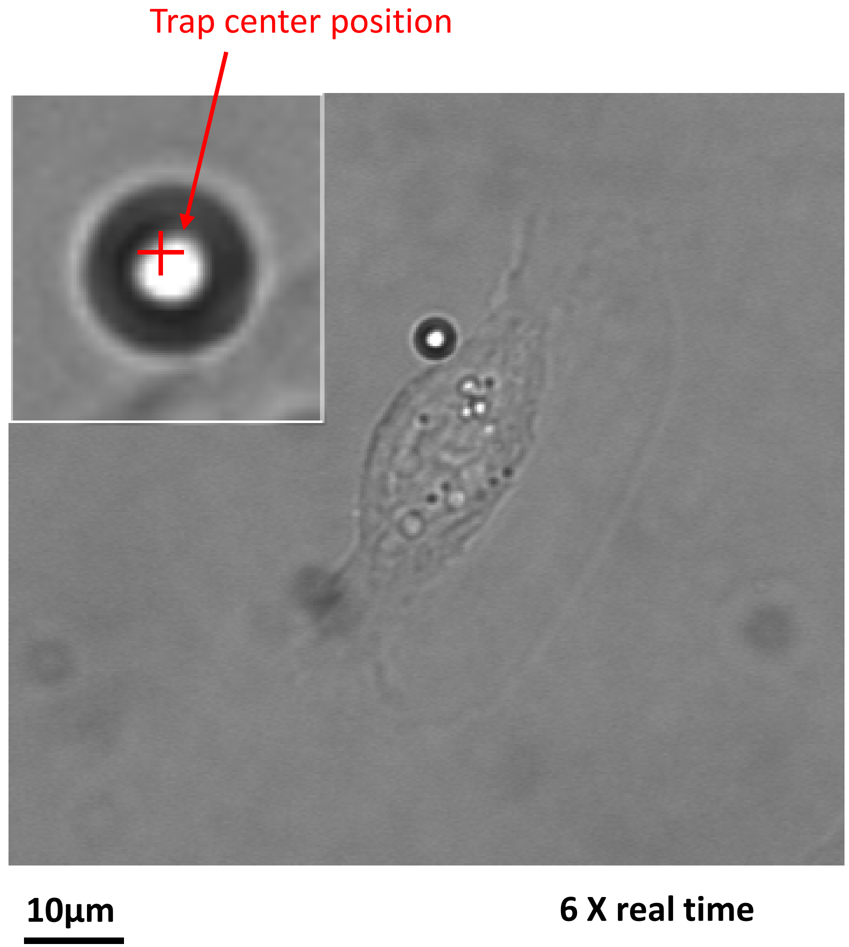
$$T = T_m + \gamma = \frac{F_{tether}^2}{8B\pi^2}$$

F_{tether} tether force
B membrane bending modulus
T apparent membrane tension

Tether pulling experiments in keratocytes

Tether force is calculated from bead displacement





A conA-coated bead is attached to a motile keratocyte. Cell movement (at ~0.5µm/s) leads to tether formation.

Movie: Arnon Lieber

Thanks!

Technion lab:

- Enas Abu-Shah
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Michael Kozlov (Tel-Aviv Univ.)