Mechanics of Contractile Actomyosin Networks

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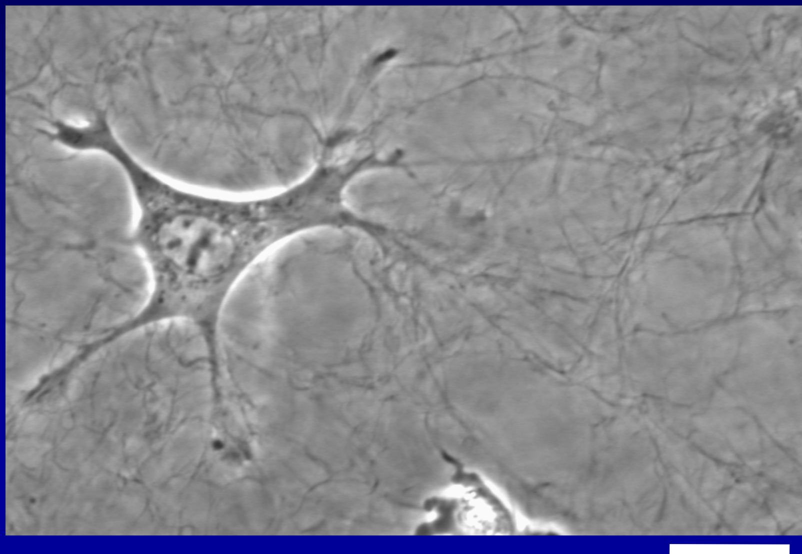
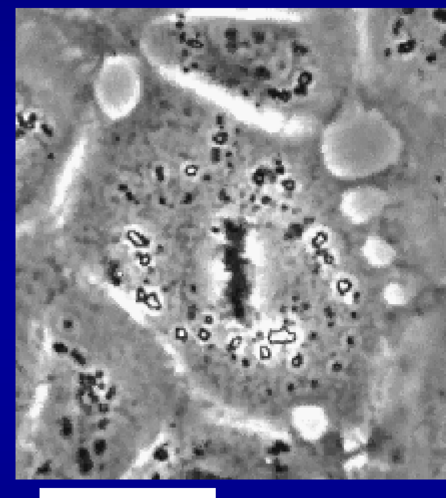
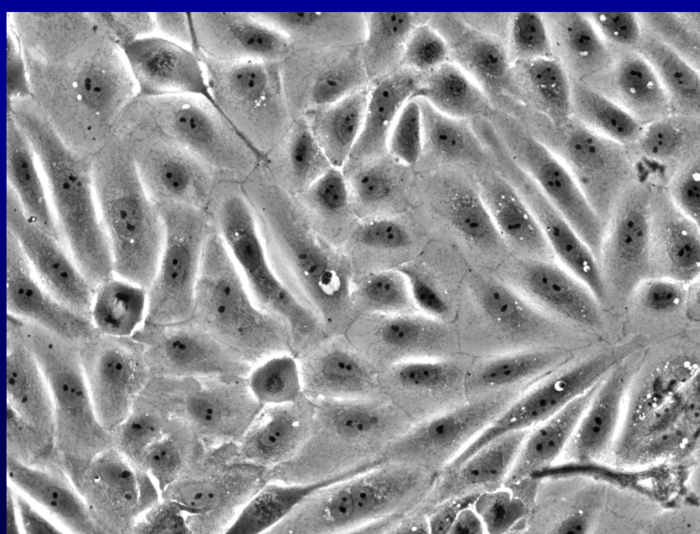
Cells in Our Body are Mechanical Machines



Time scale: 20 min- 5 hr



10 μm

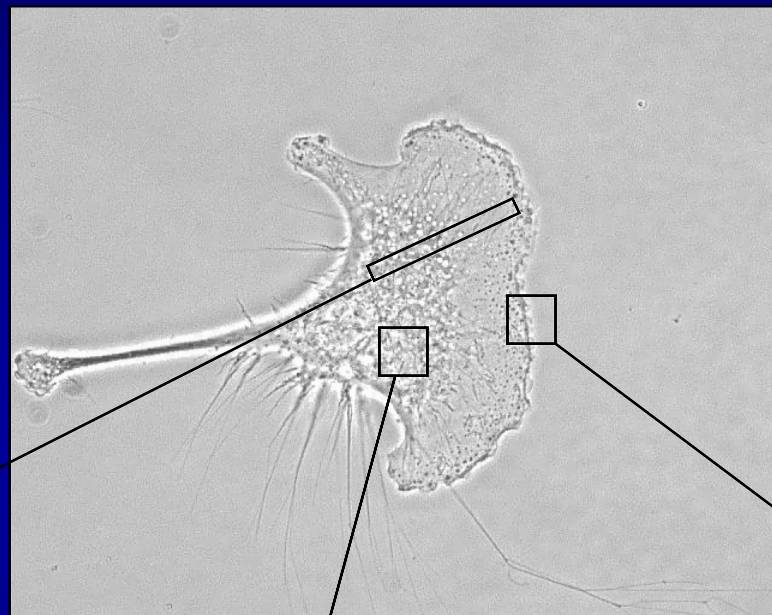


Move, Apply Force to Environment
Change Shape
Withstand force to create sheets
…….much more

DW2

DW1

Cytoskeleton determines physical behaviors of cells



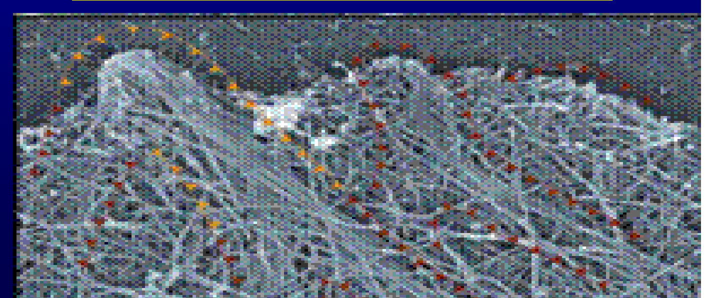
Contractile Bundles

Network

Protrusive Network

(Cramer)

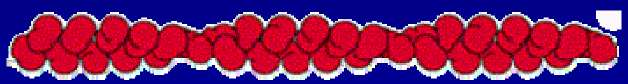
(Hartwig)



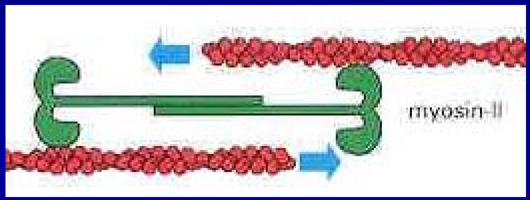
(Svitkina)

Cyotskeletal Building Blocks

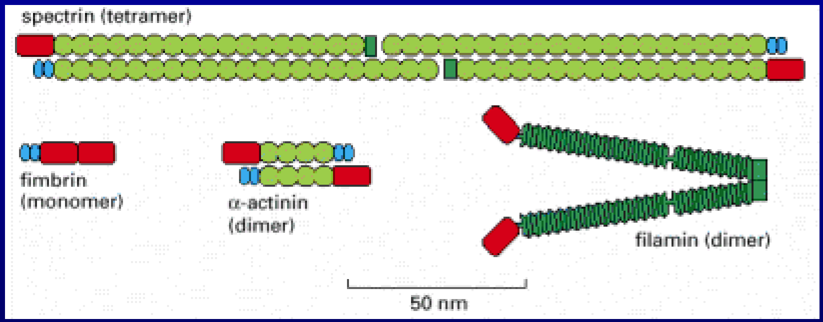
Polymers



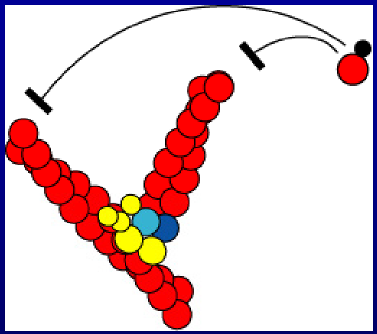
Molecular Motors



Polymer Cross-linkers

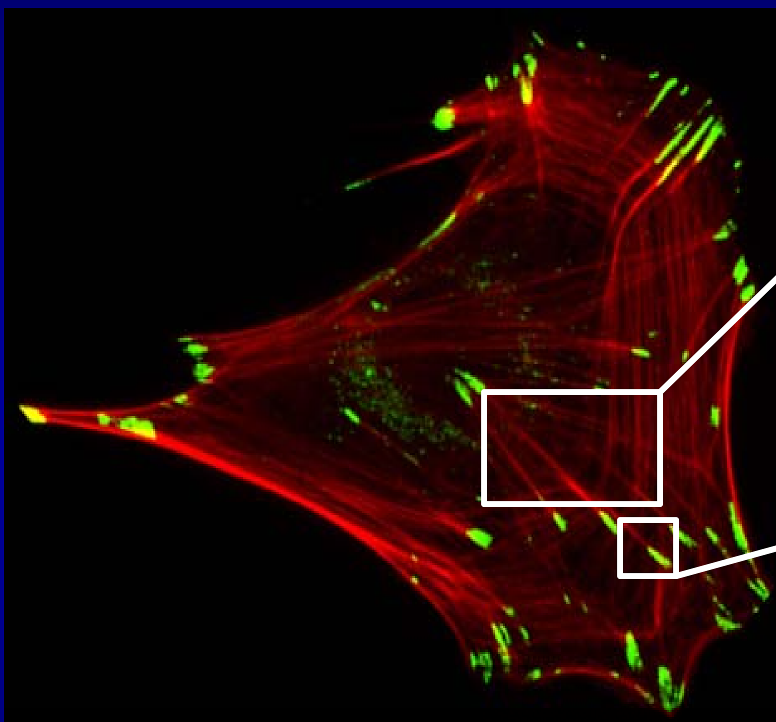


Polymer Assembly



How do these proteins build (active) materials required for
cytoskeletal machines?

Contractile Actomyosin Cytoskeleton



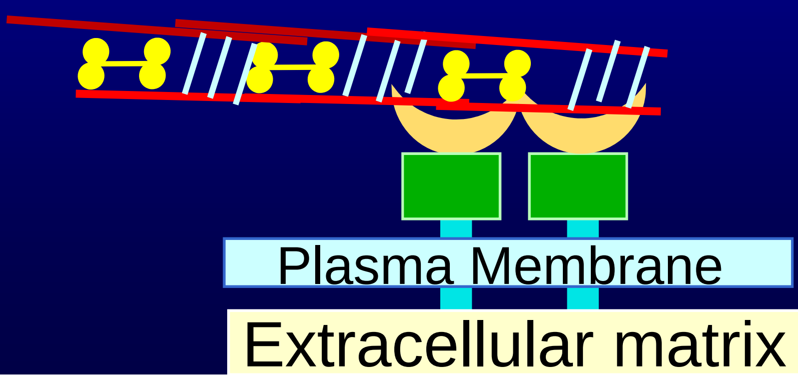
Actin Filaments

Myosin-II Motors

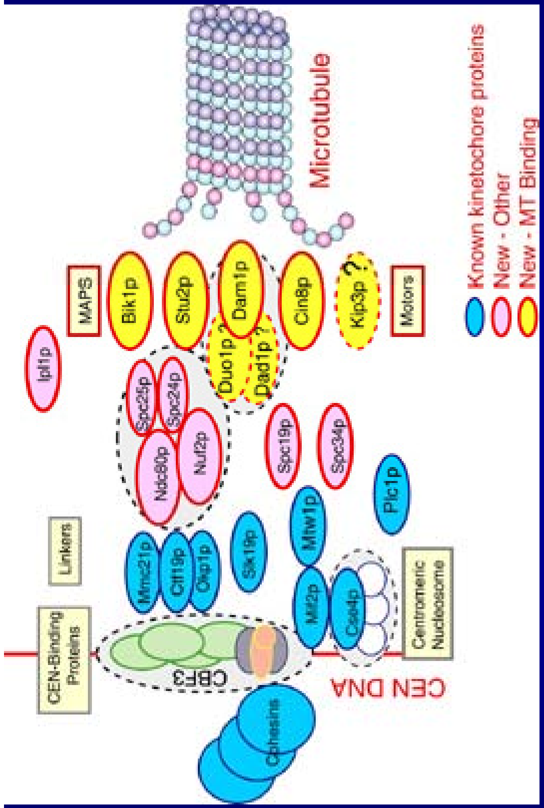
α-actinin crosslinks

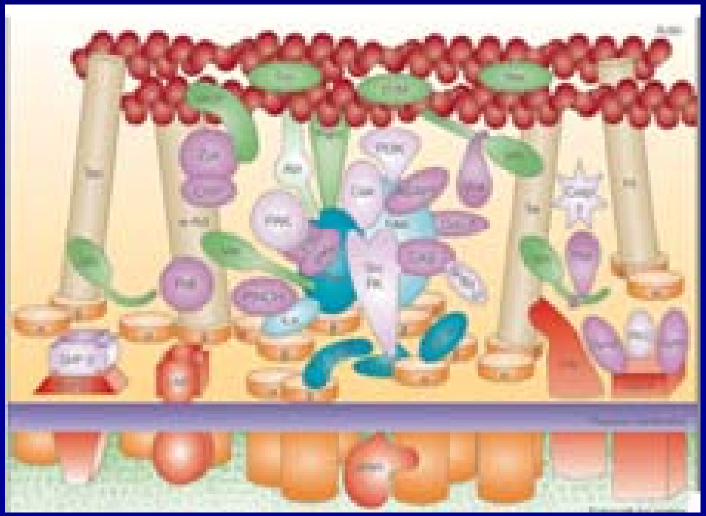
Focal Adhesions

>100 structural/signaling proteins



Focal Adhesions and kinetichores



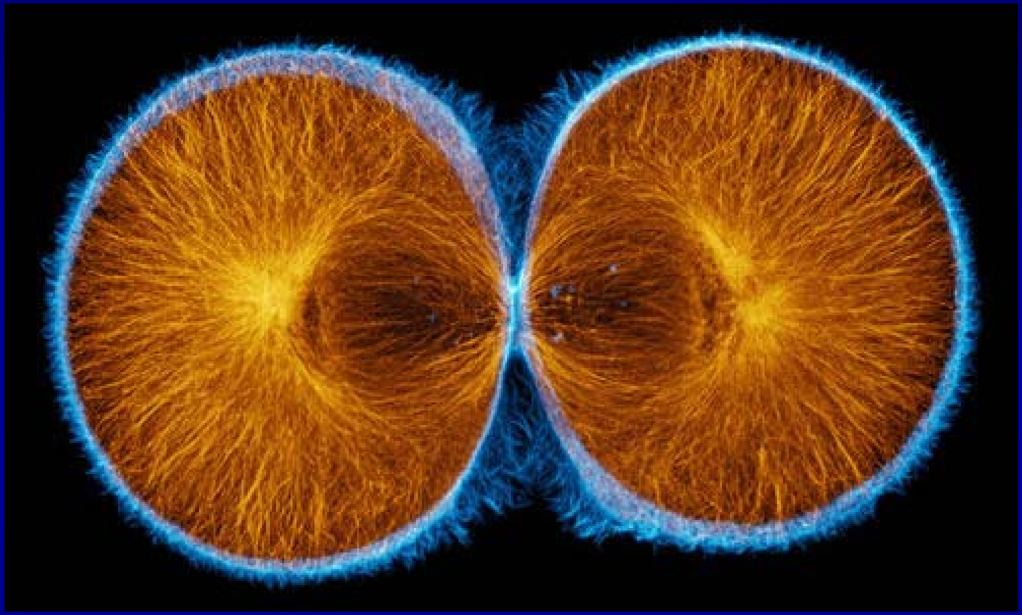


Dynamic polymer

Complicated Attachment (tension sensing?)

Force application

Actomyosin Cortex

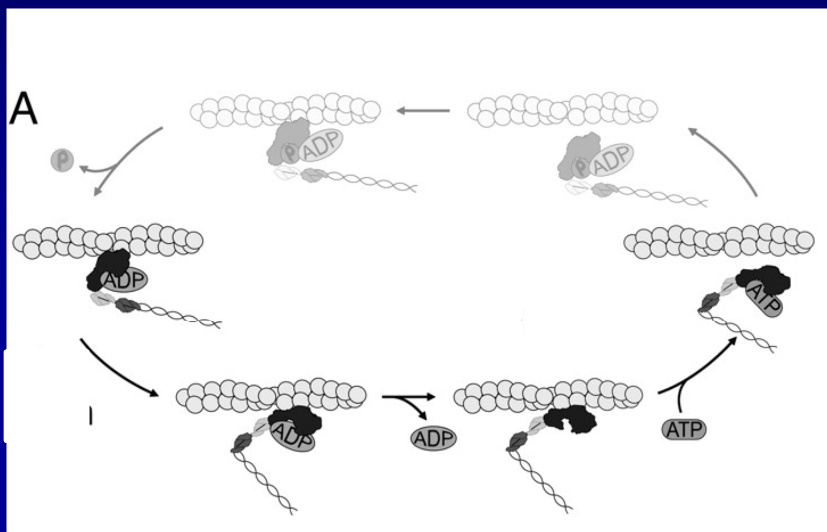


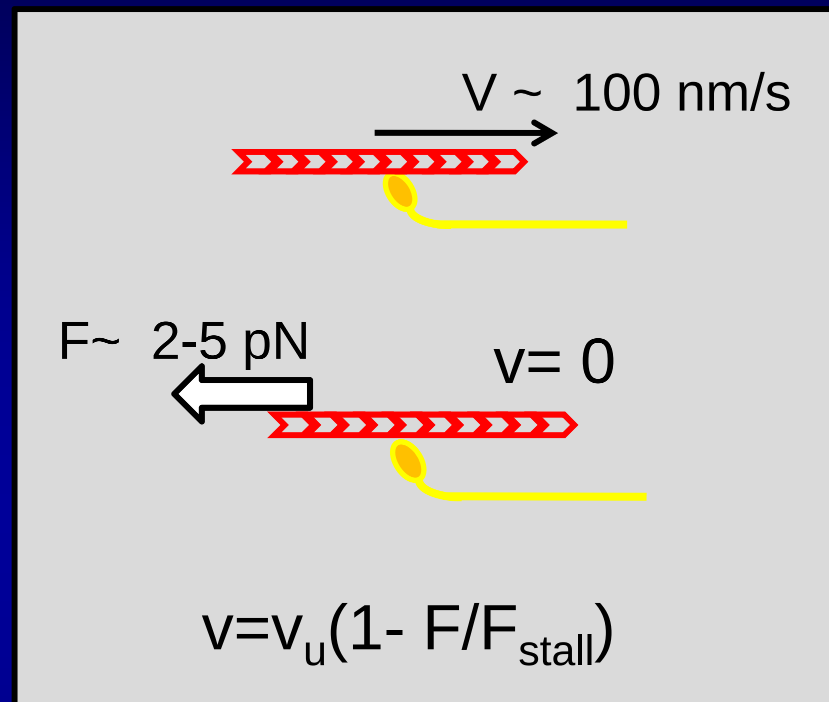
Different boundary conditions from adherent cortex

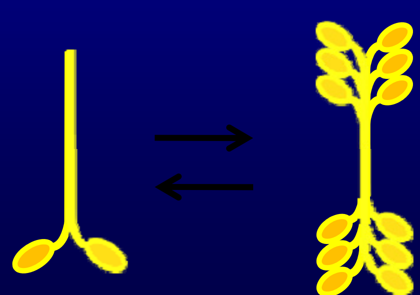
Are there common rules of self-assembly of actomyosin that can govern Self-assembly?

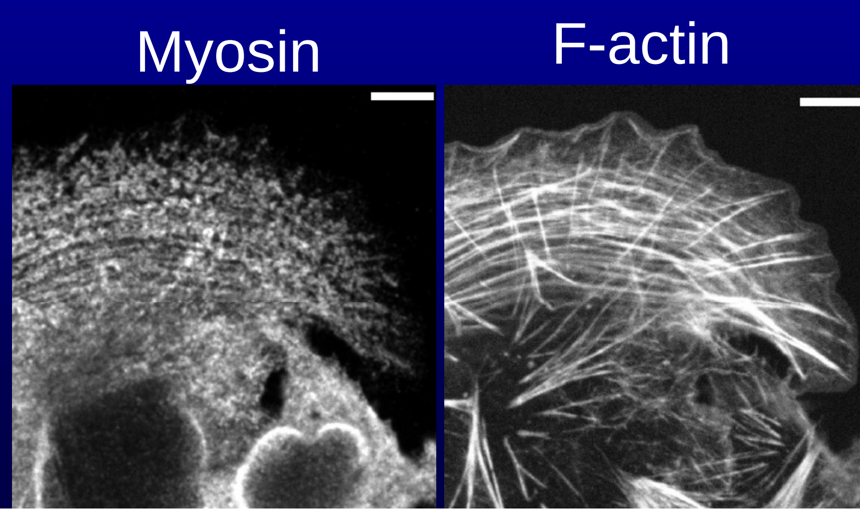
Any cell that doesn't have a wall likes to have this cortex

Myosin Mechanochemistry and Assemblies

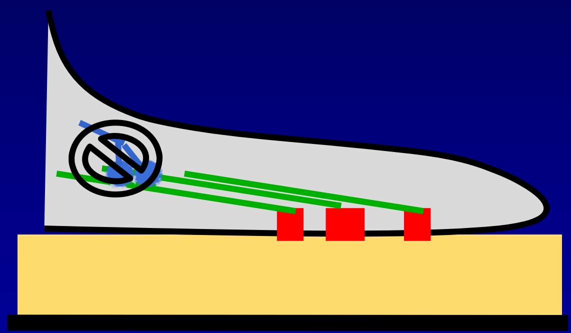


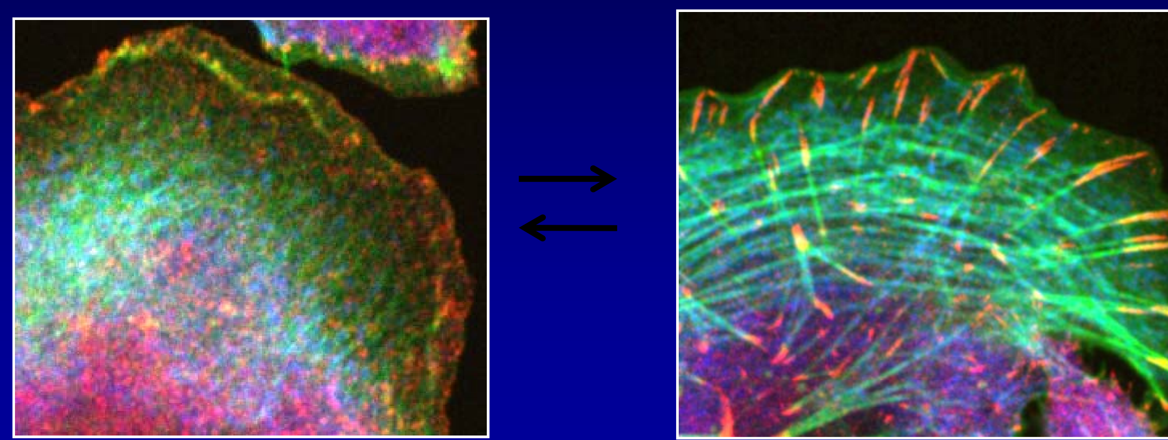






Feedback between Forces and Actin Cytoskeleton

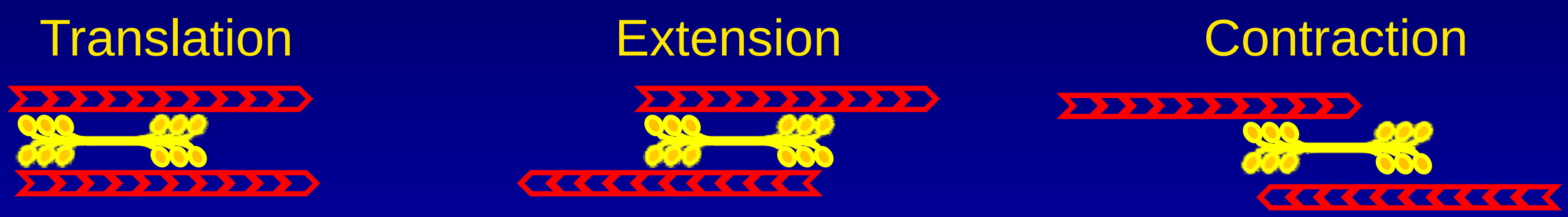




ECM Stiffness	Soft (.6-3kPa)	Stiff (3-15 kPa)
Internal Tension (Myosin activity)	Low	High
F-actin	Meshwork	Bundles
Traction Force	Low	High

Impacts: Proliferation, Migration, Gene Expression, Differentiation,

What regulates self assembly of motor/filament bundles?

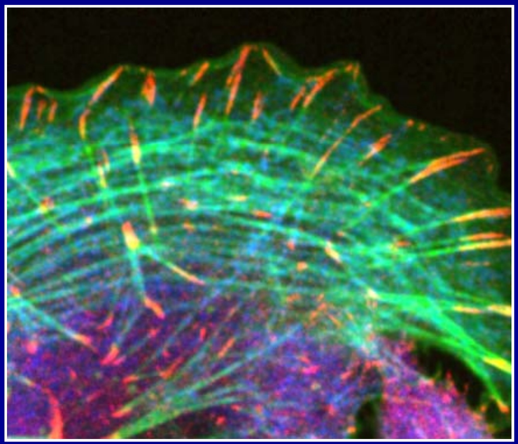


- What regulates whether you get an extending or contractile bundle
- Is passive crosslinker required for contraction?
- How does structure depend on tensile forces?

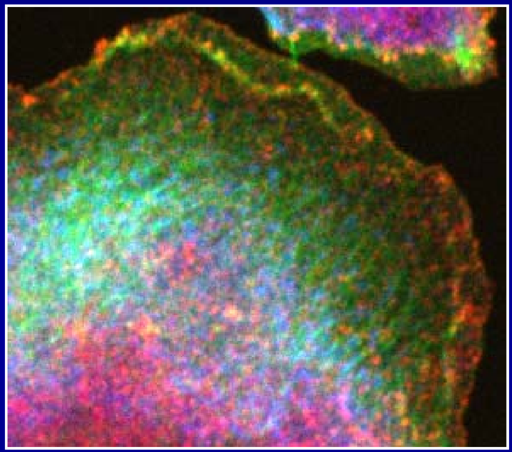
What rules govern self-assembly of actomyosin bundles?
- In cells
- In purified proteins

Myosin-II ATPase inhibitor dynamically regulates contractile phenotype

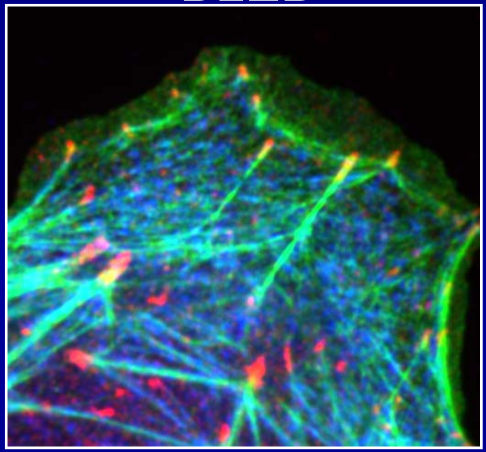
Control



+ 25 μM Blebbistatin
30 min

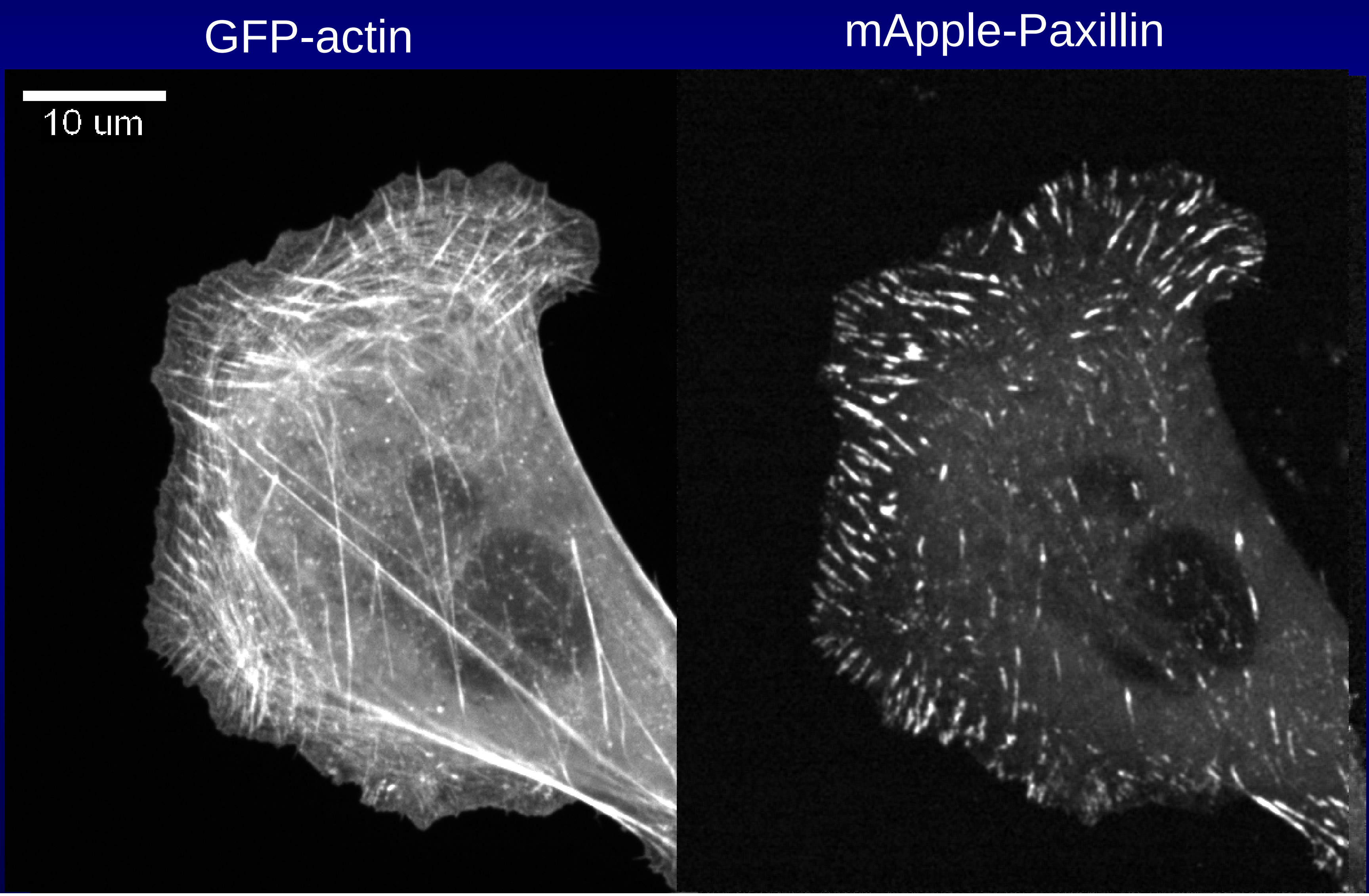


15 min after Removing BLEB



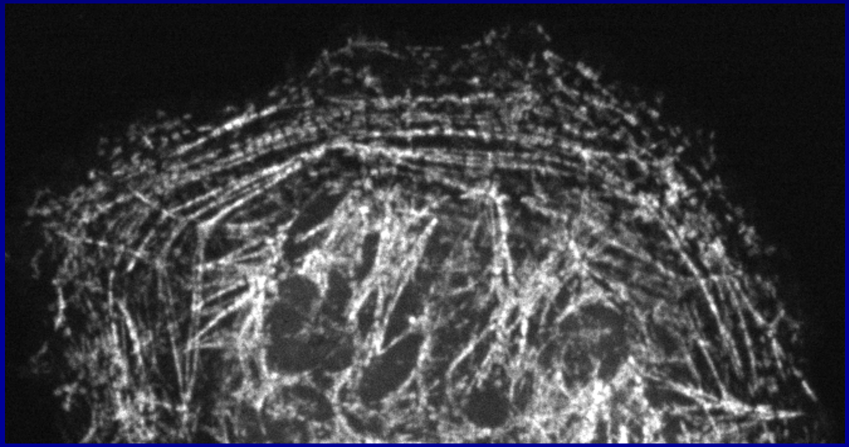
Actin
Paxillin
Myosin Light Chain

Blebbistatin Washout Drives Self-Assembly of Bundled Actin

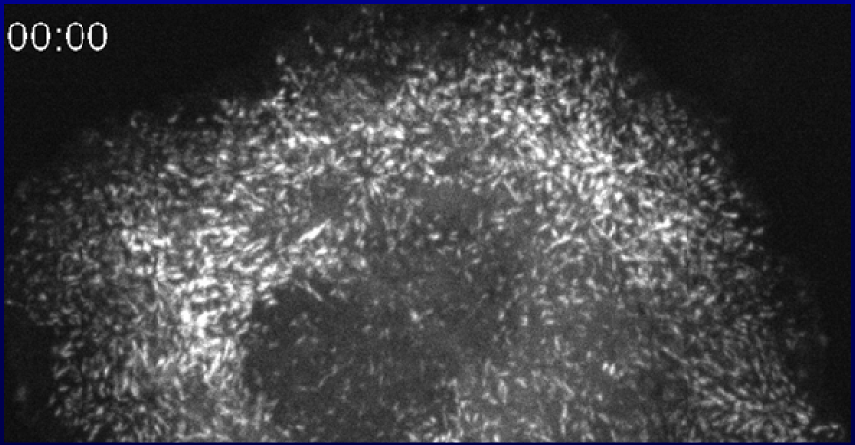


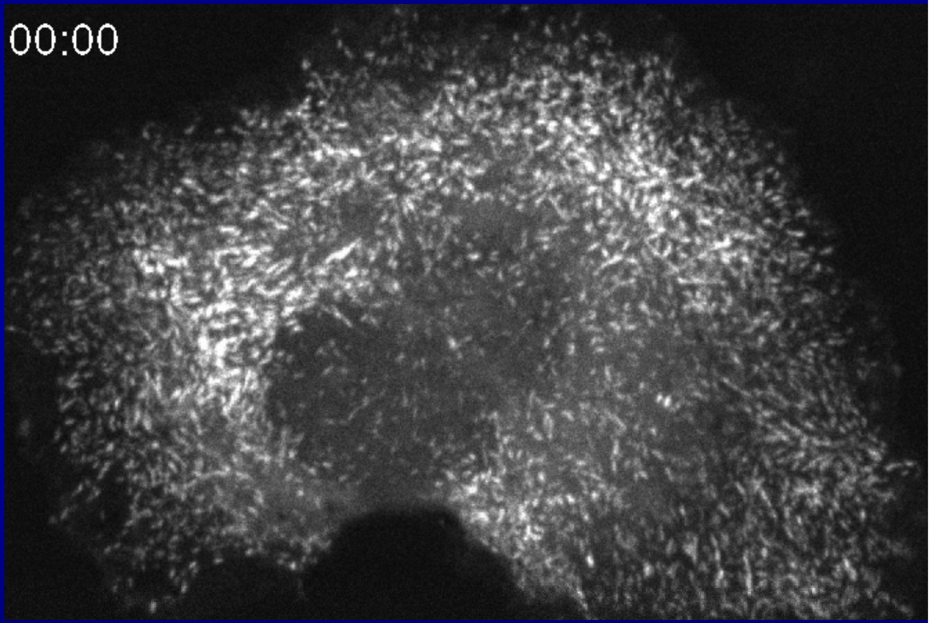
Myosin Dynamics Drive Remodelling

Control



+ Blebbistatin

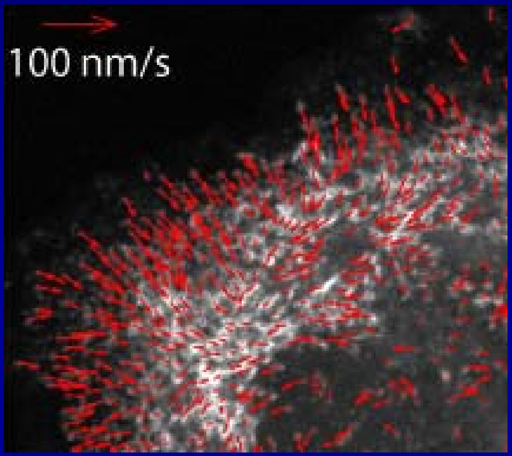


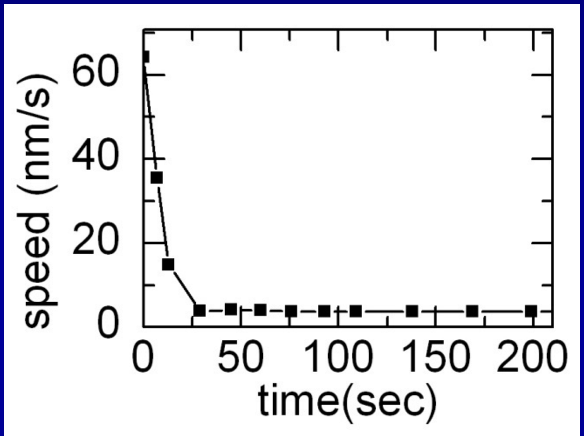


GFP-myosin light chain

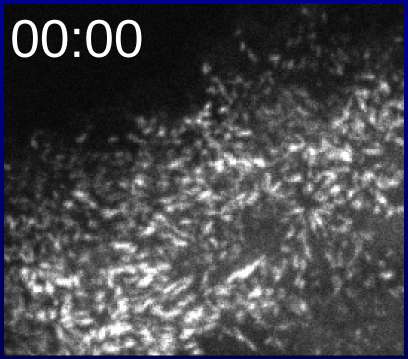
Dynamics and Structure during Remodeling

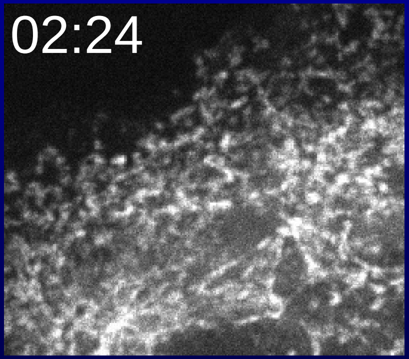
I. Short time scale Motion Dynamics

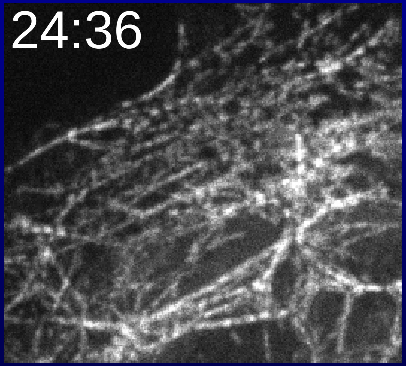




II. Long time scale bundle formation



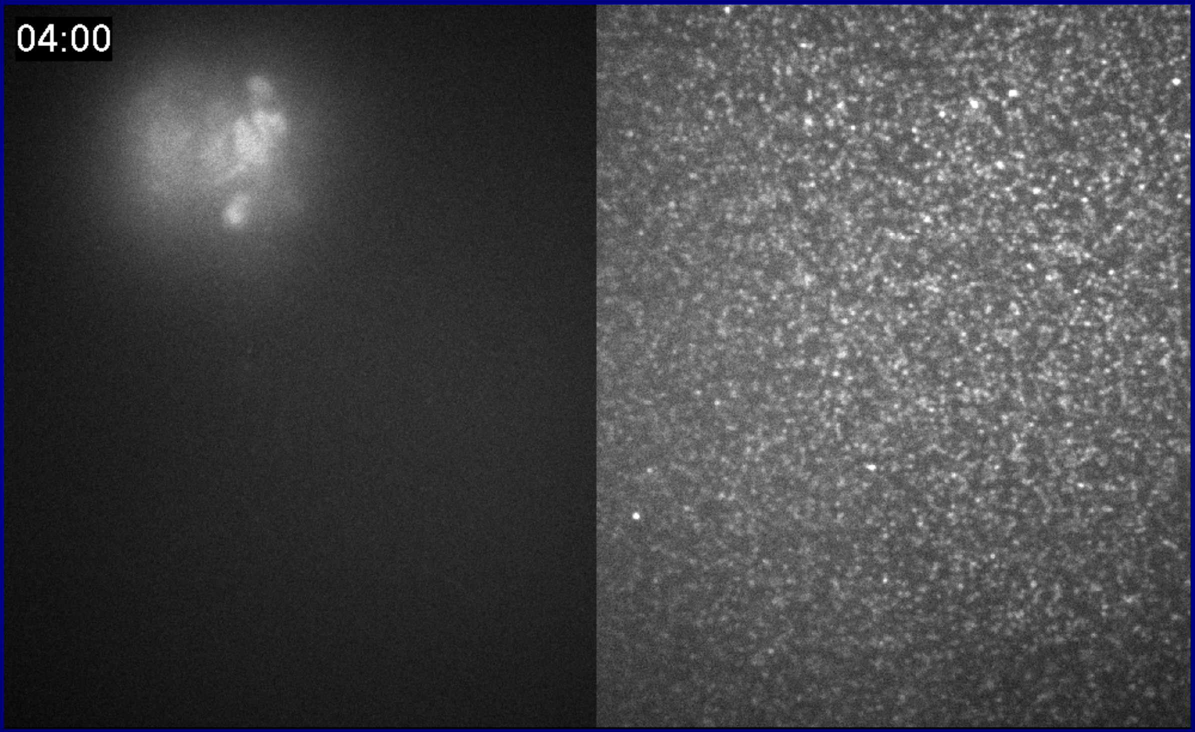


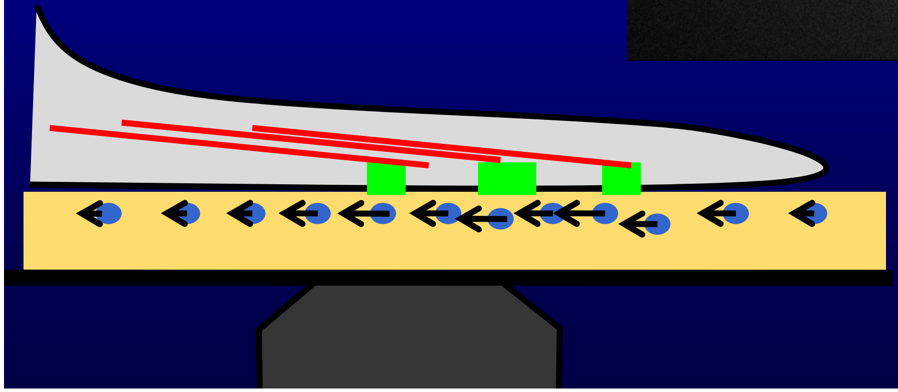


How do these relate to tension build up?

High Resolution Traction Force Microscopy

GFP-actin | Cy5 beads

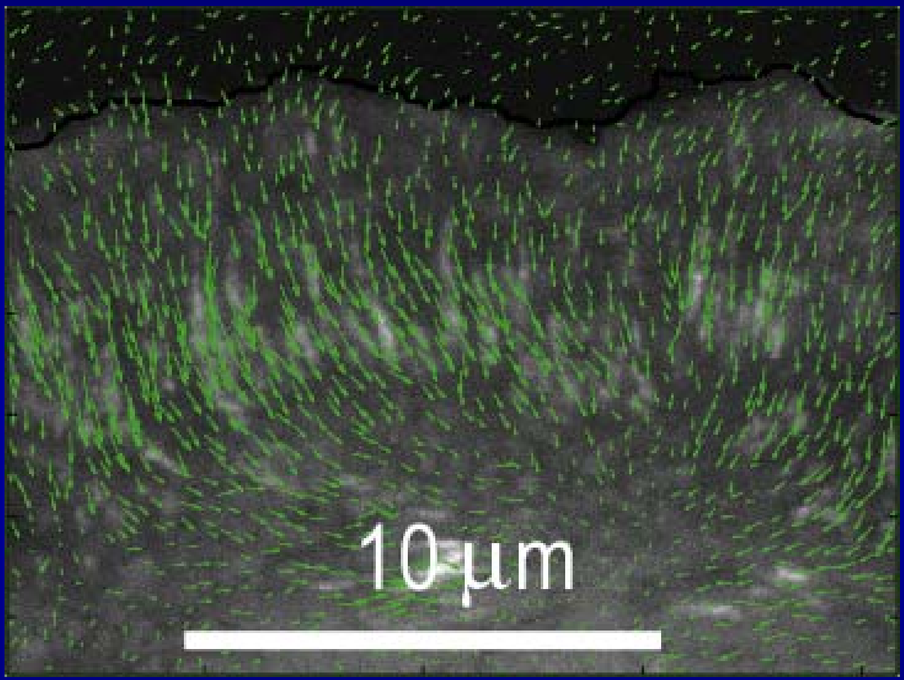




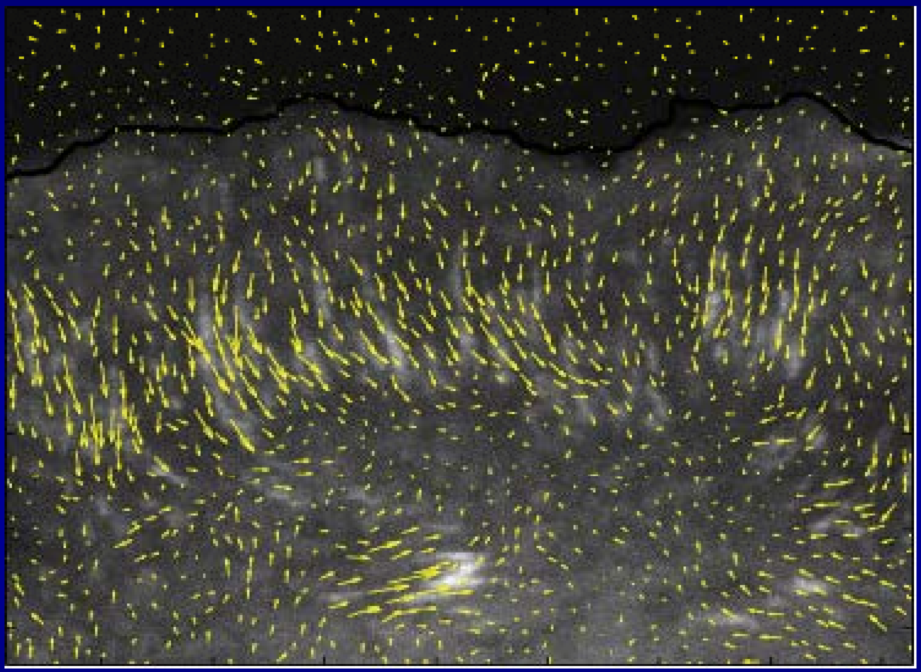
10-20 μm thick polyacrylamide gel
40 nm far red latex spheres

Traction Stress Reconstruction

Strain Field, U



Stress Field, F



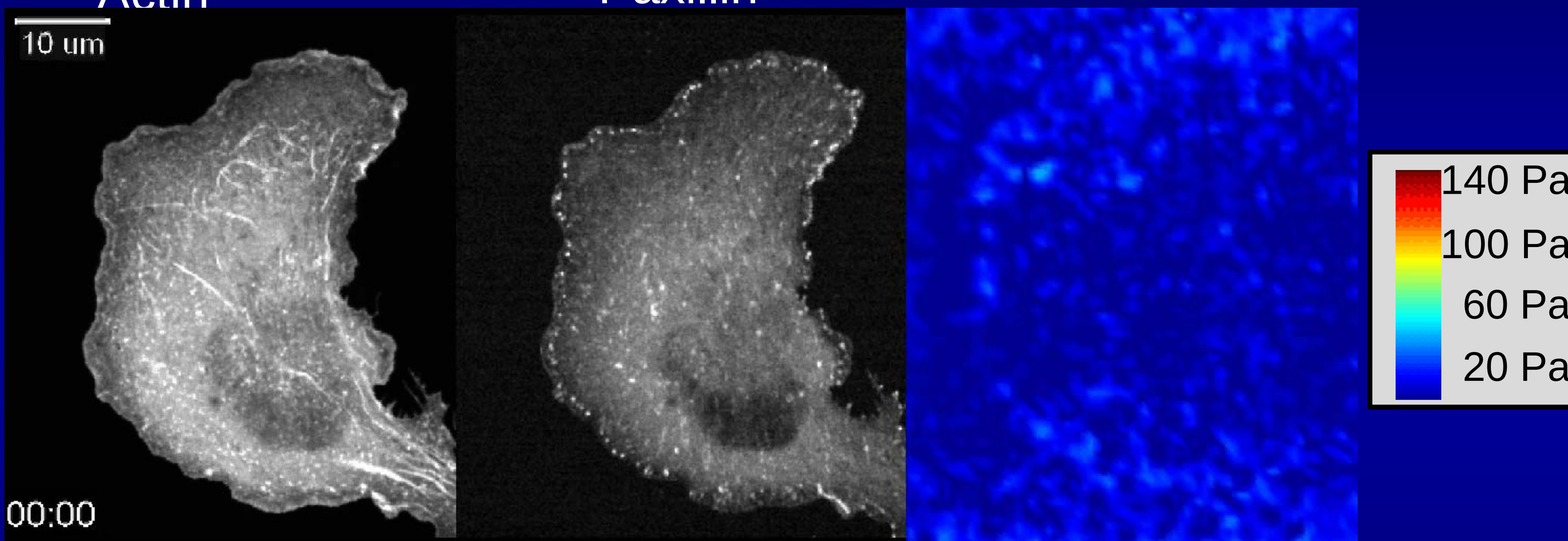
$$U_{\alpha k} = G_{\alpha\beta ki} F_{\beta i}$$

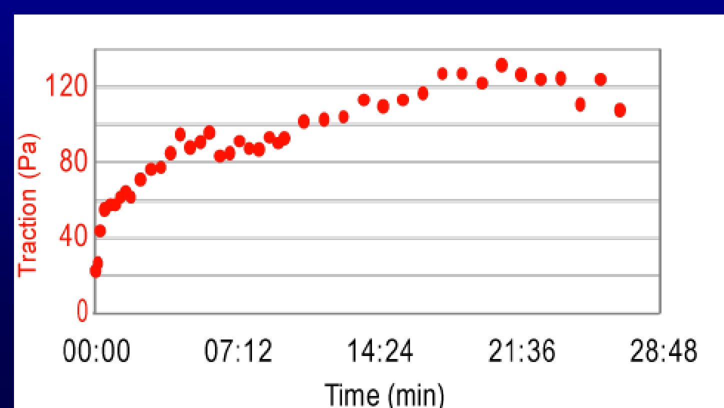
$$F_{\beta i} = \operatorname{argmin} \left(\left\| U_{\alpha k} - G_{\alpha\beta ki} F_{\beta i} \right\| + \lambda \left\| F_{\beta i} \right\| \right)$$

Sabass, Gardel et. al. Biophys. J 2007

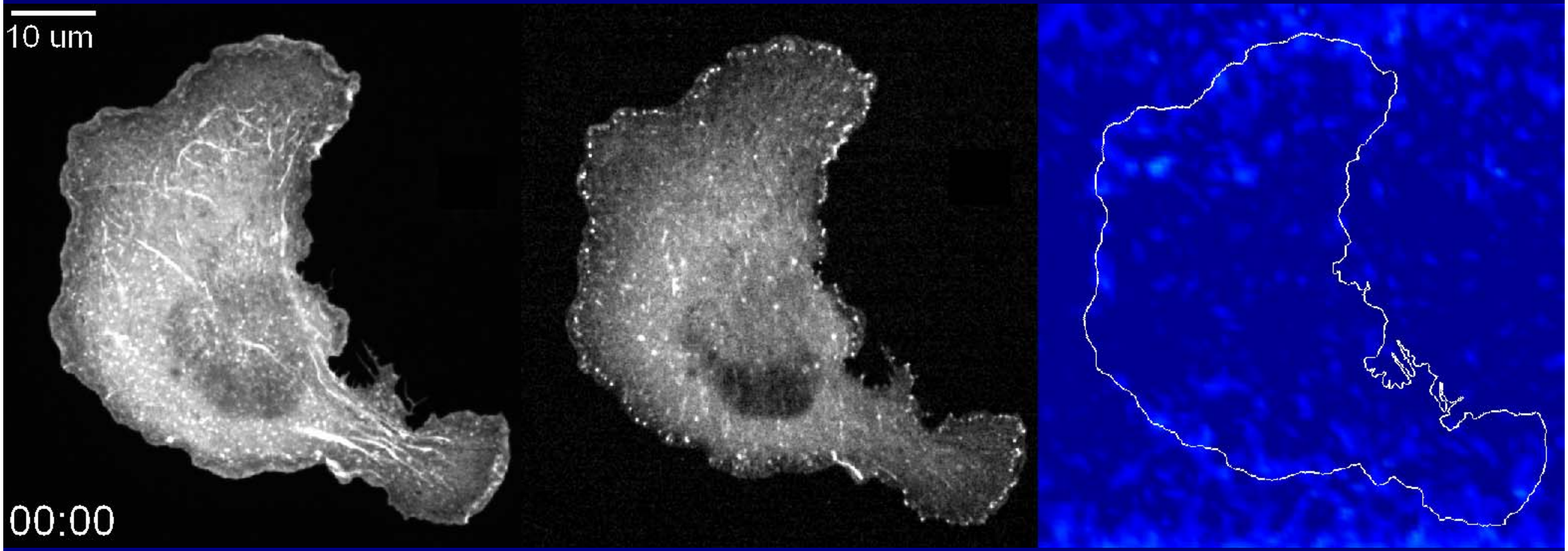
Traction Stress Increases as Bundles Form

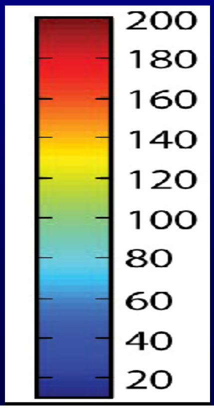
Actin Paxillin Traction Stress



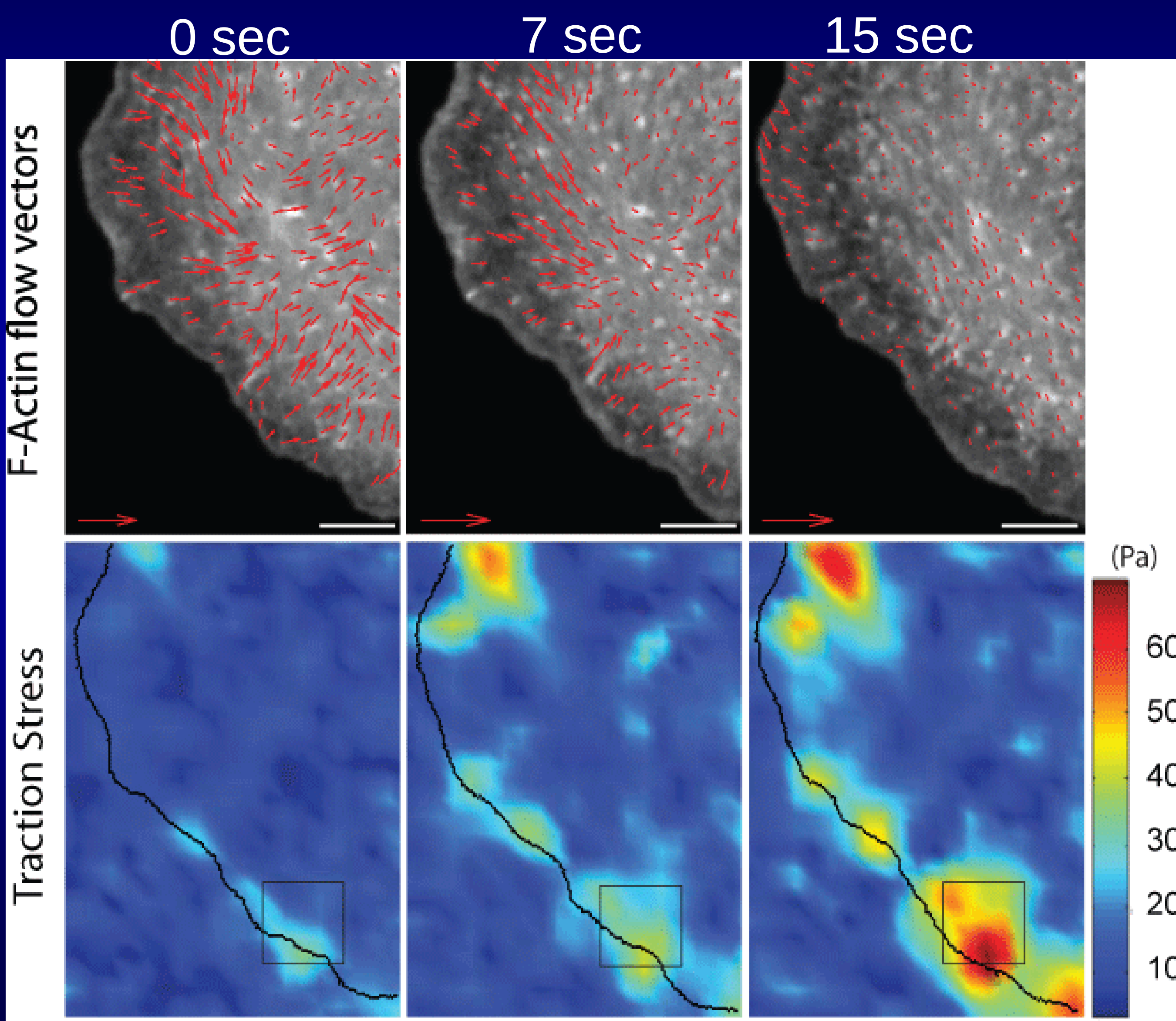


Minimal Change in Structure during first 30 seconds

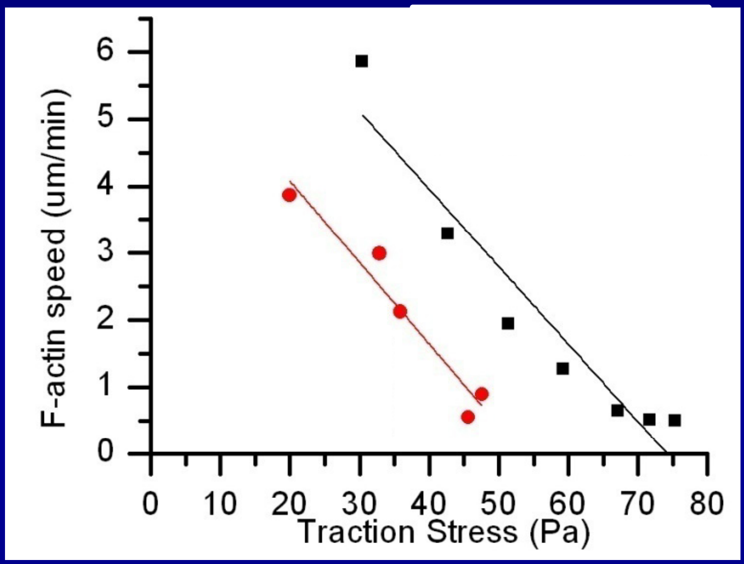




Traction builds and F-actin flow slows



Force-velocity of lamellar F-actin Cytoskeleton



1. Myosin II mechanochemistry

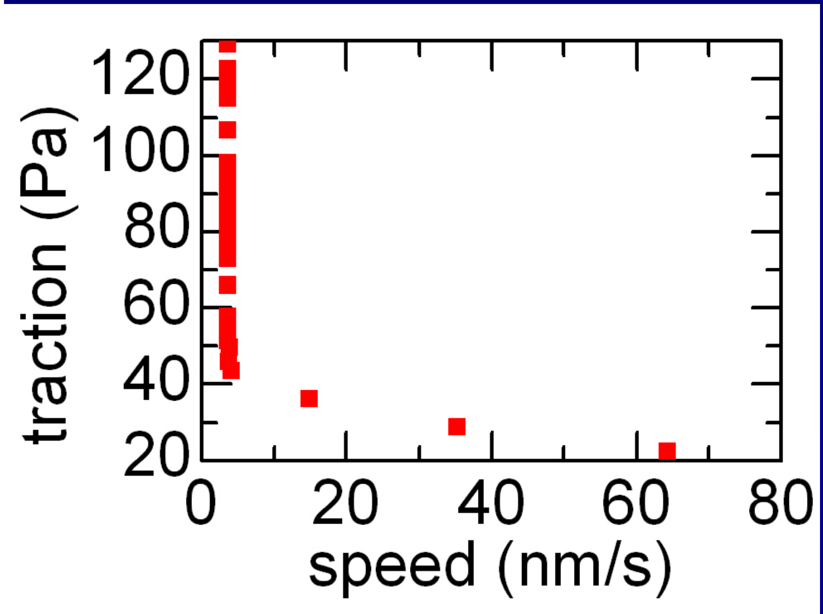
$$v_{ACT}=v_u(1- \sigma_{trc}/\sigma_{stall})$$

	Cell	In Vitro
v_u	108 nm/s	~100 nm/s
σ_{stall}	50 Pa	
F_{stall}	5 pN	2 pN

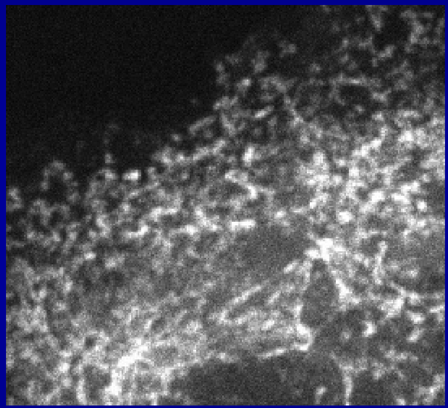
2. Active Liquid Crystal Models

(Julicher/Prost)

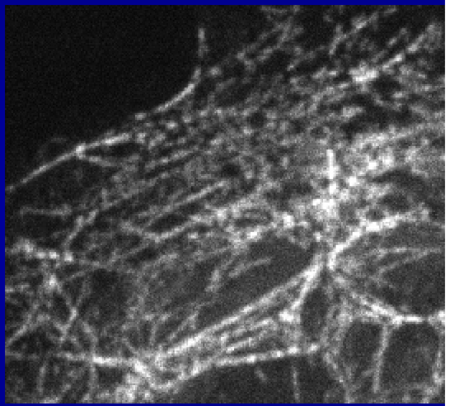
Nonlinear Force-velocity Relationship



02:24



24:36

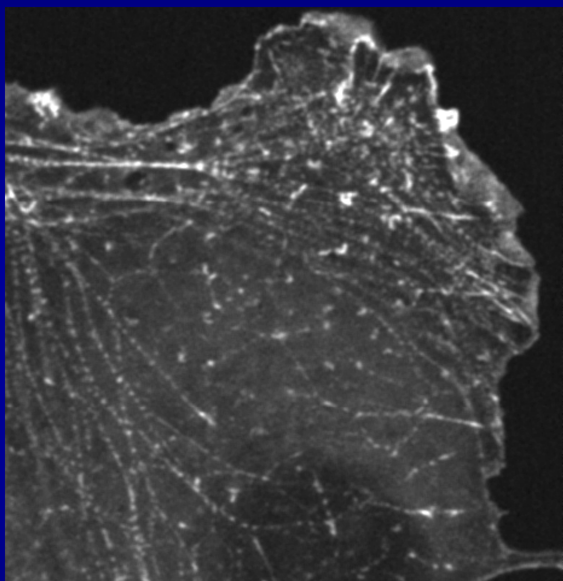


How do changes in structure correlate to tensile stress build up?

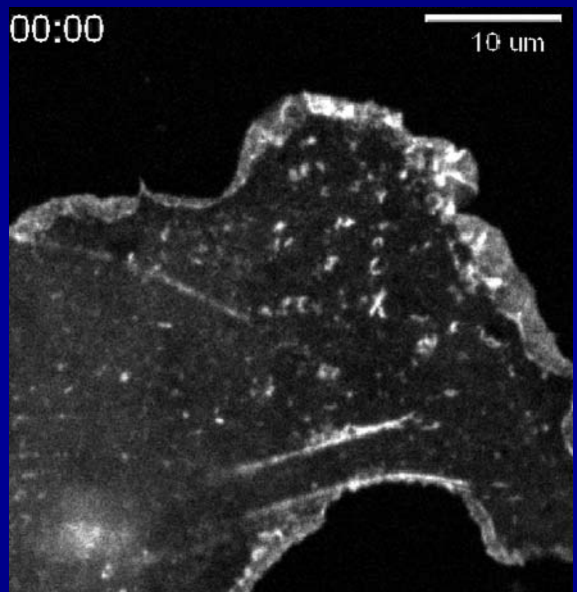
Order Parameter?

α-actinin tracks formation of bundled F-actin

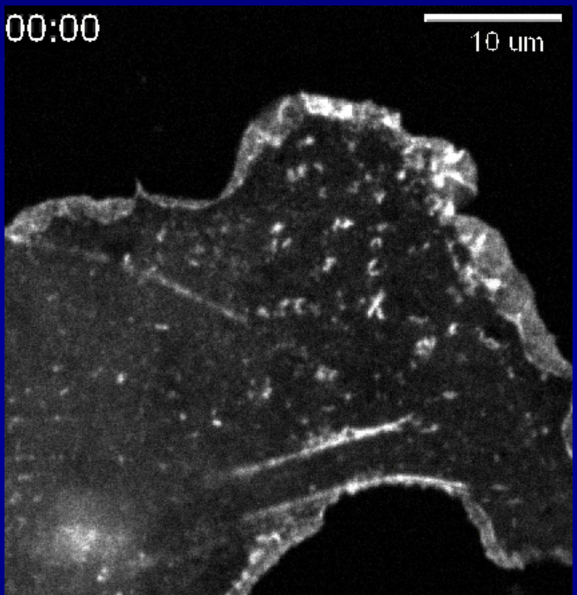
Control

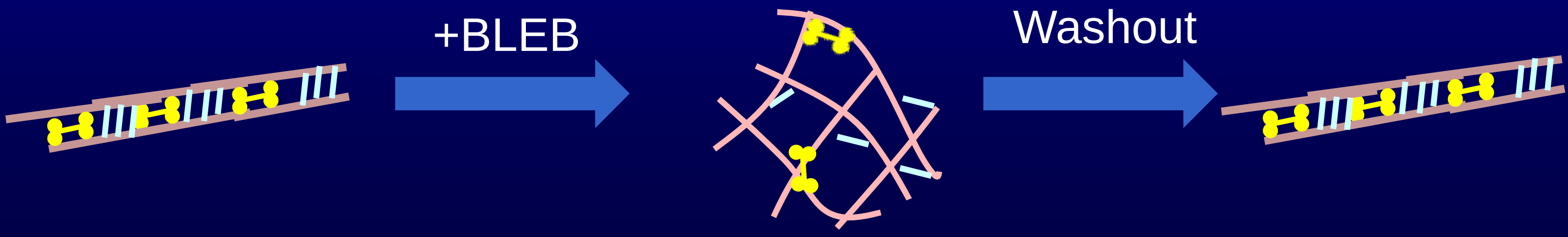


+ Blebbistatin

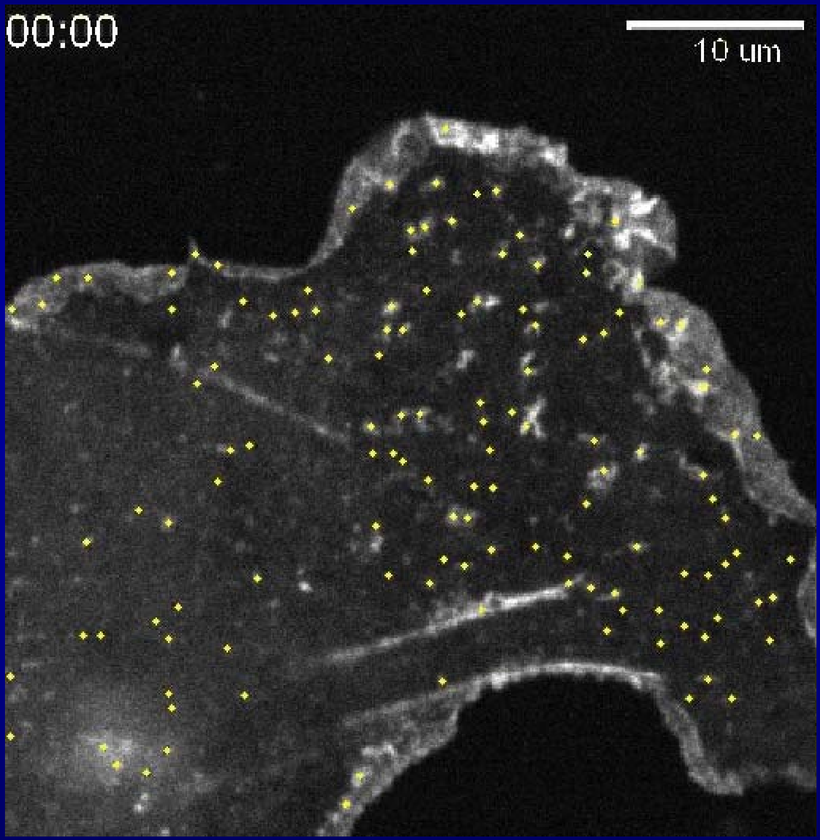


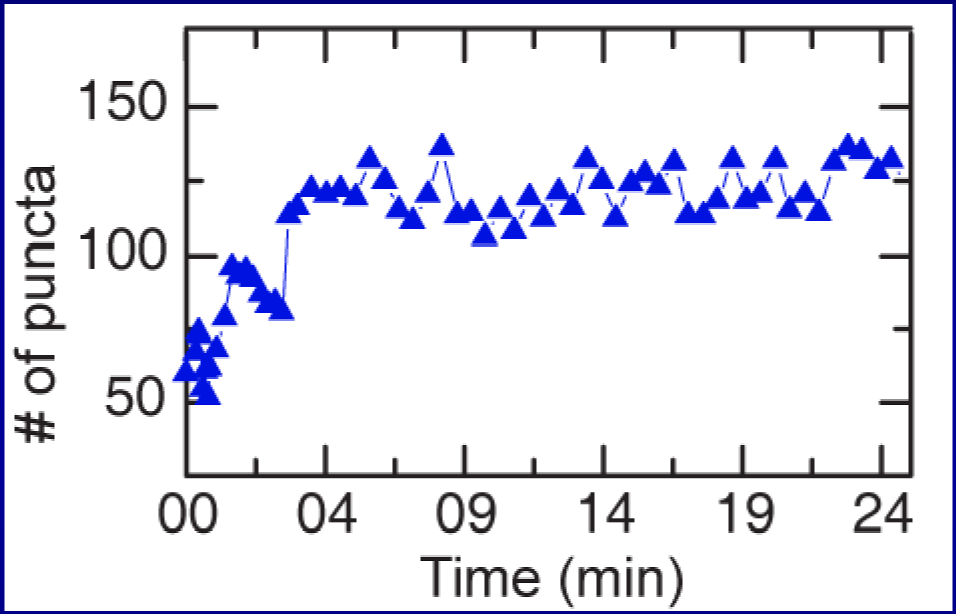
Washout



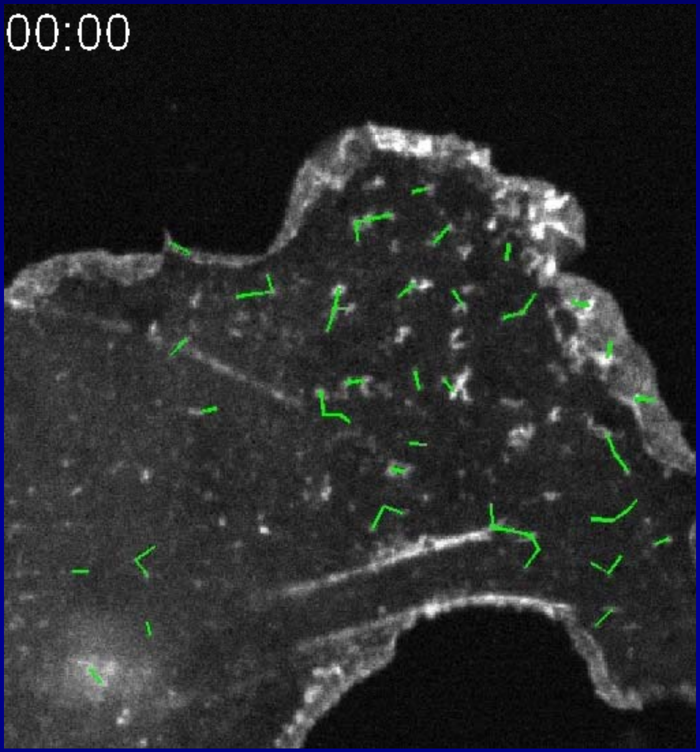


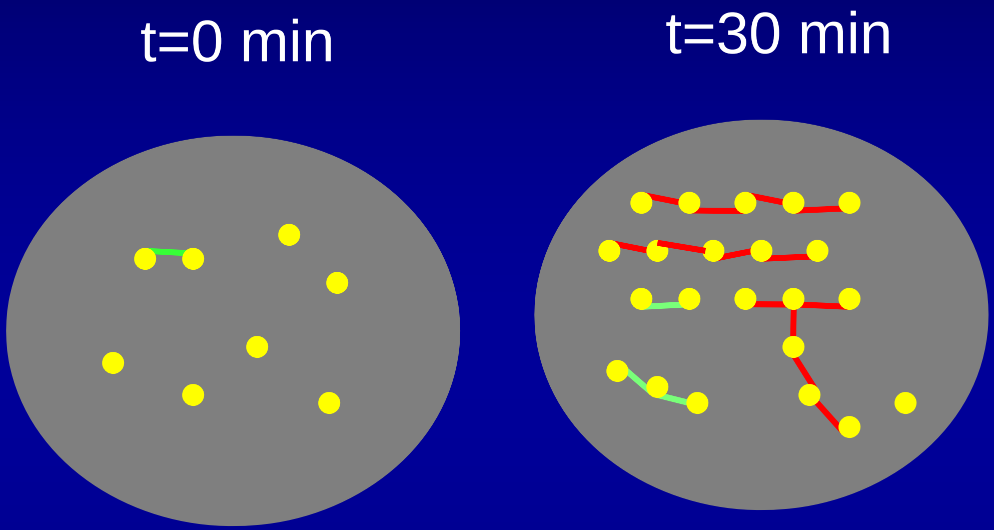
Density of bundled F-actin Increases





Large clusters of closely packed α-actinin puncta form as traction builds

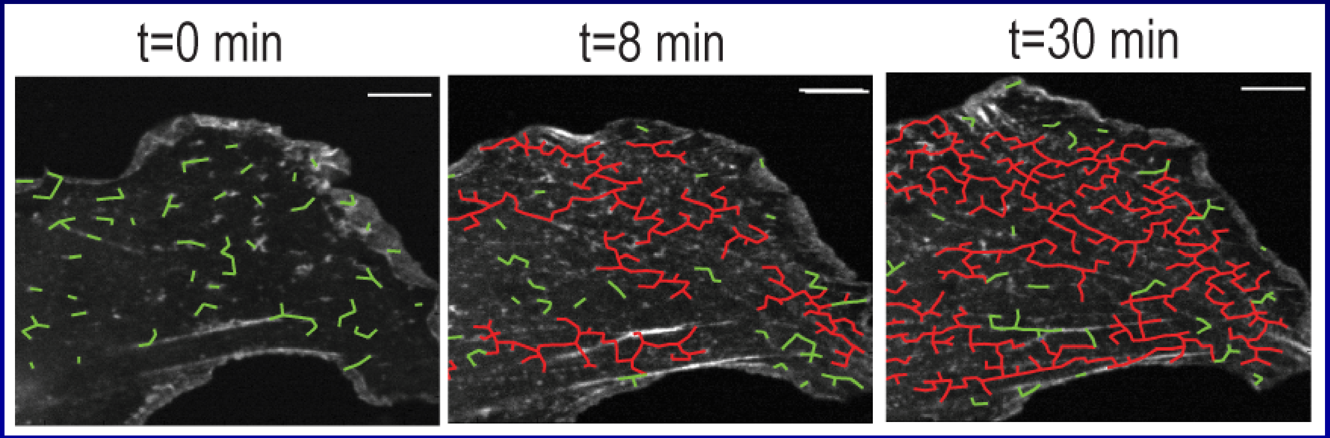


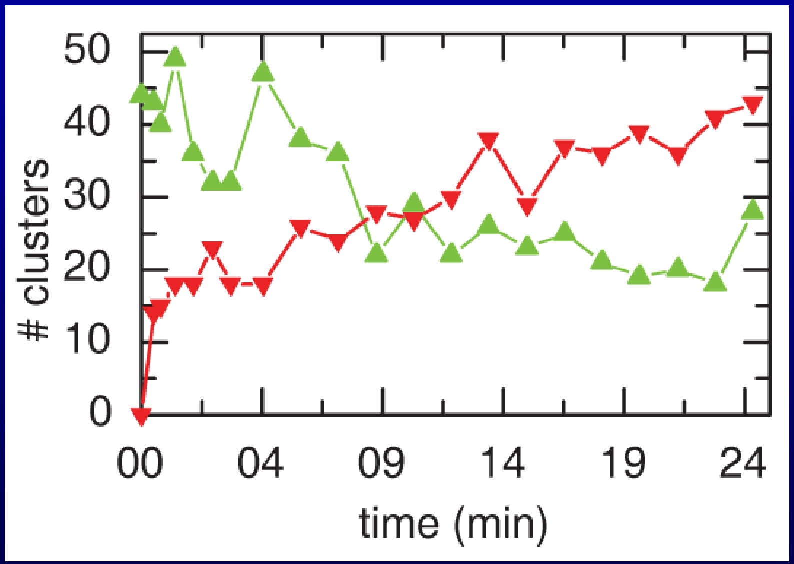


SMALL CLUSTERS

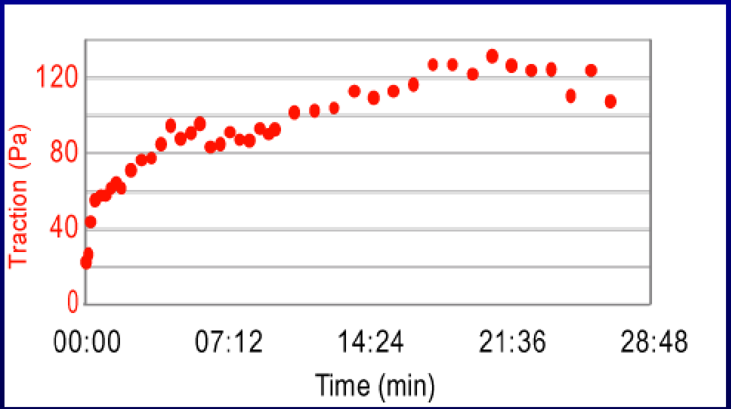
LARGE CLUSTERS

Assembly of space-spanning contractile bundles Correlate to traction build up





Traction



Physical Parameters guiding cellular traction

- <u>Low tension</u>

  Homogeneous F-actin network

  Force is inversely correlated to movement of F-actin + myosin

- <u>High tension</u>

  Remodeling into contractile actomyosin bundles

  Force is proportional to contraction

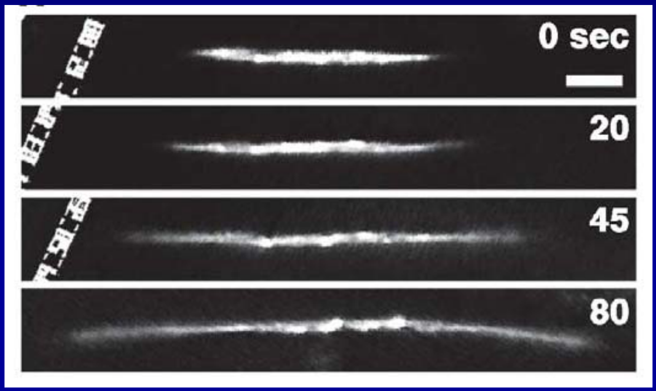
Substrate Sensing

  Interplay between internal drag and strain sensitivity of actomyosin bundles

  → Need Model System to isolate parameters

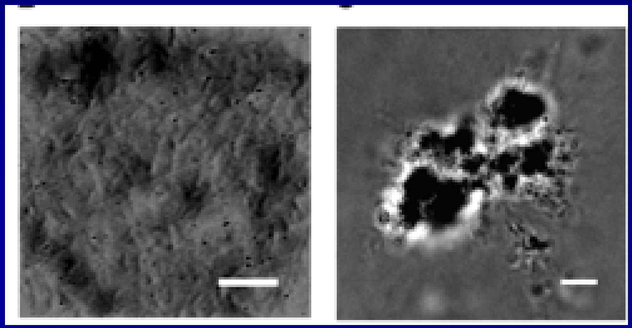
Dynamics of *in vitro* Actomyosin Bundles

Expansion



Taiguchi, 2004

Contraction



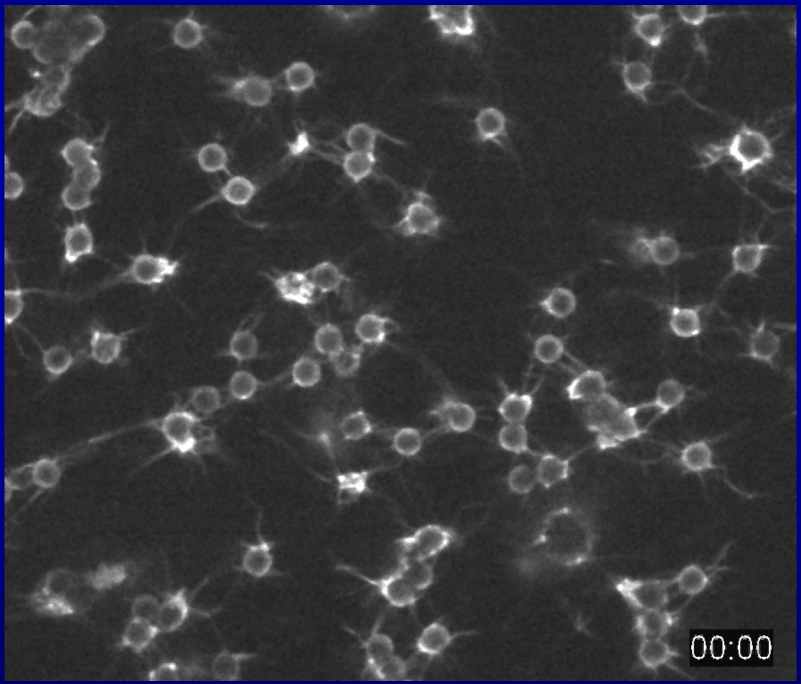
Mizuno, 2004

Passive cross-linker required for contraction of gels

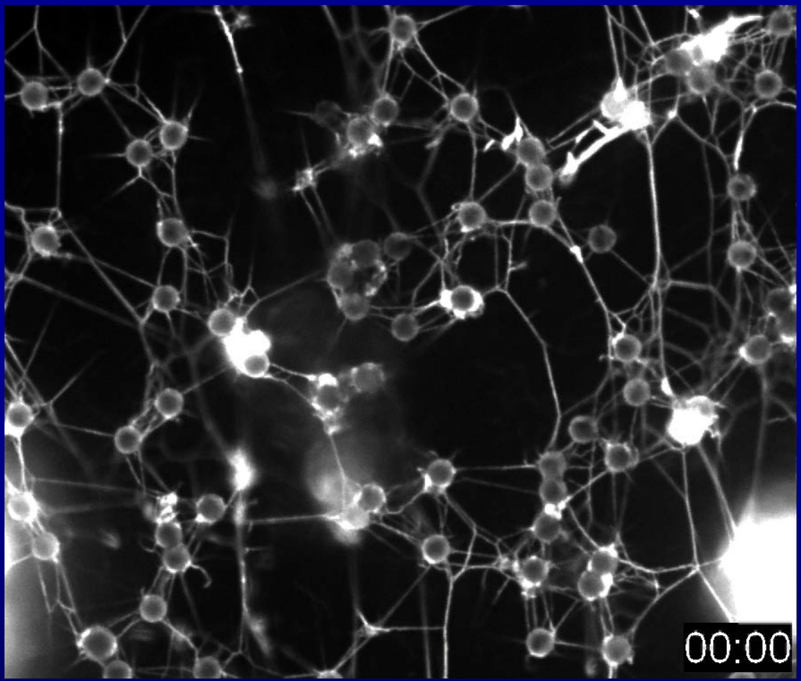
Free boundary conditions
→ stress fibers have fixed boundary conditions

Construction of *in vitro* Actomyosin Bundles

F-actin

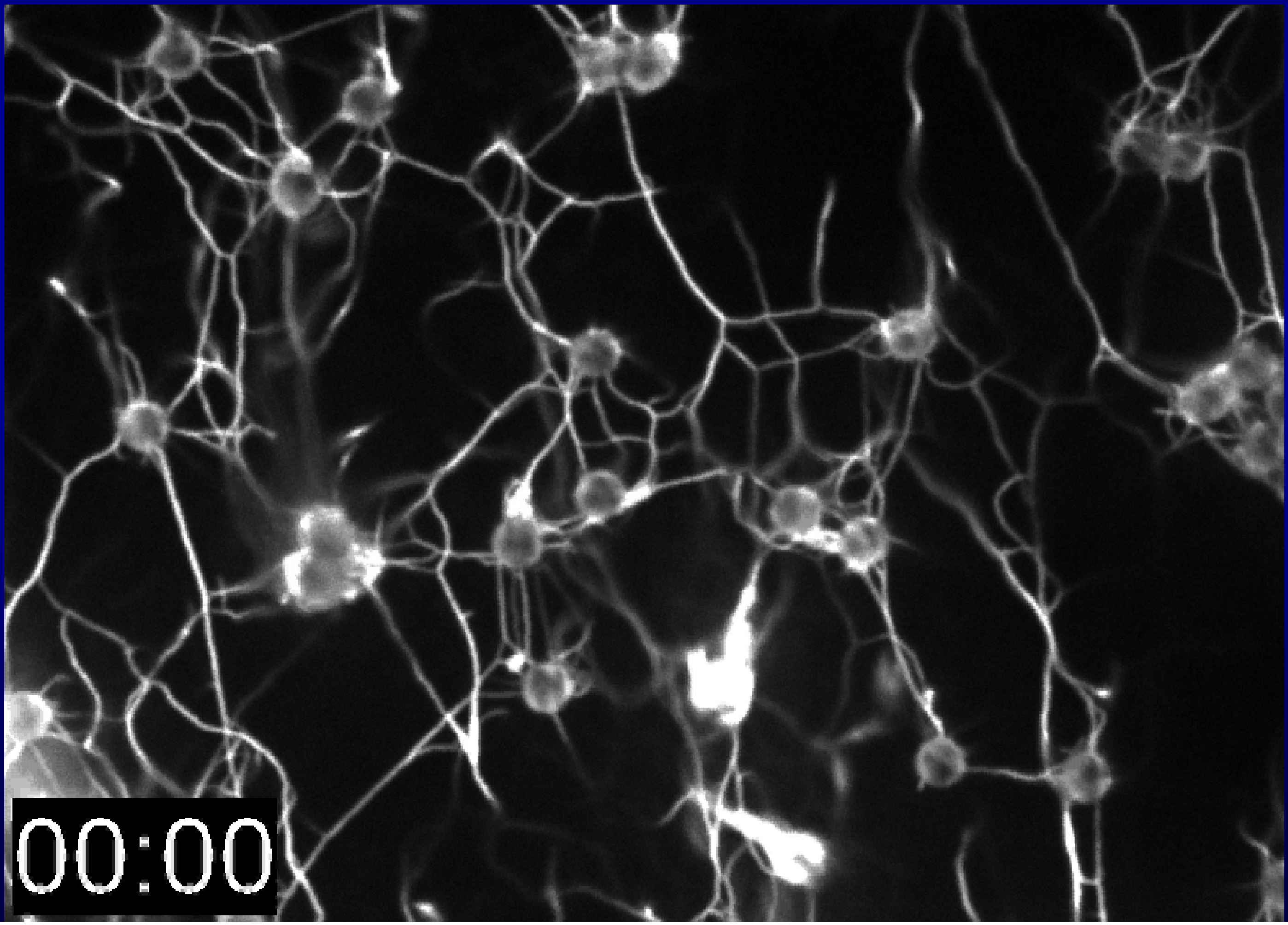


Add ADP-myosin



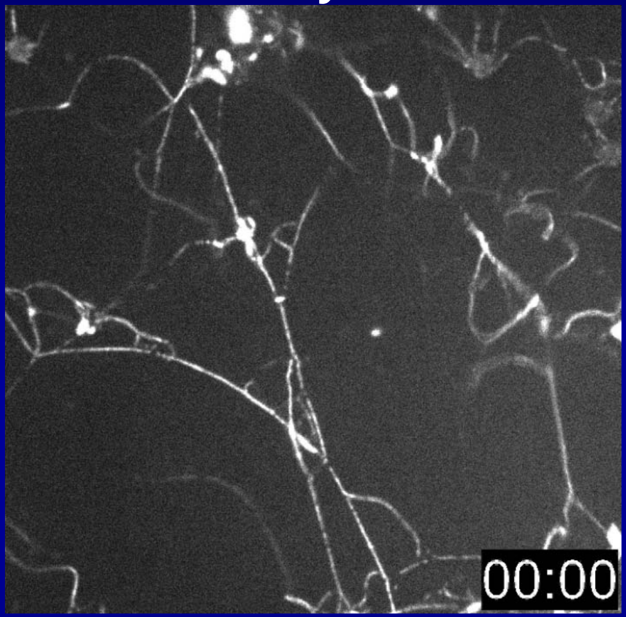
ATP-dependent remodeling

Perfuse 100 mM ATP

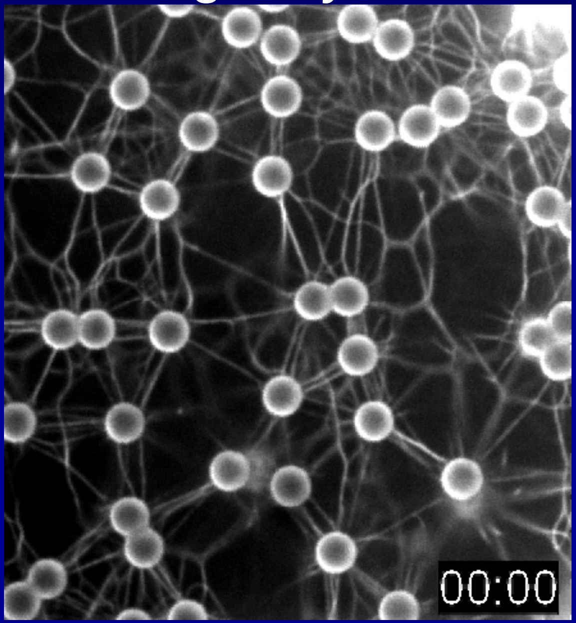


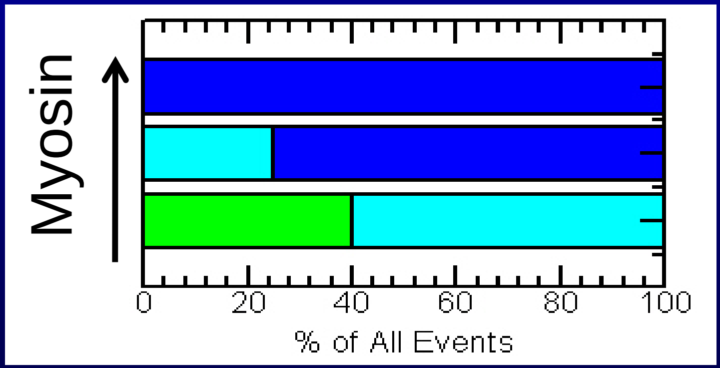
Myosin Concentration Regulates Contractility

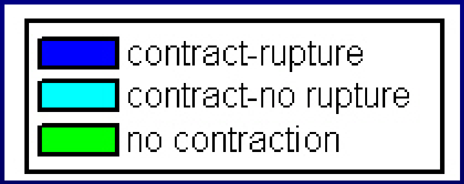
Low Myosin



High Myosin



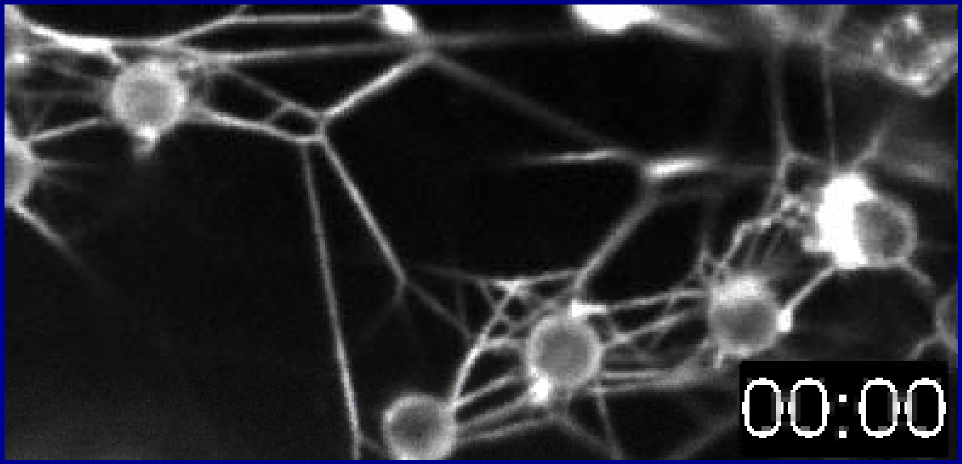




Why do these structures always contract?

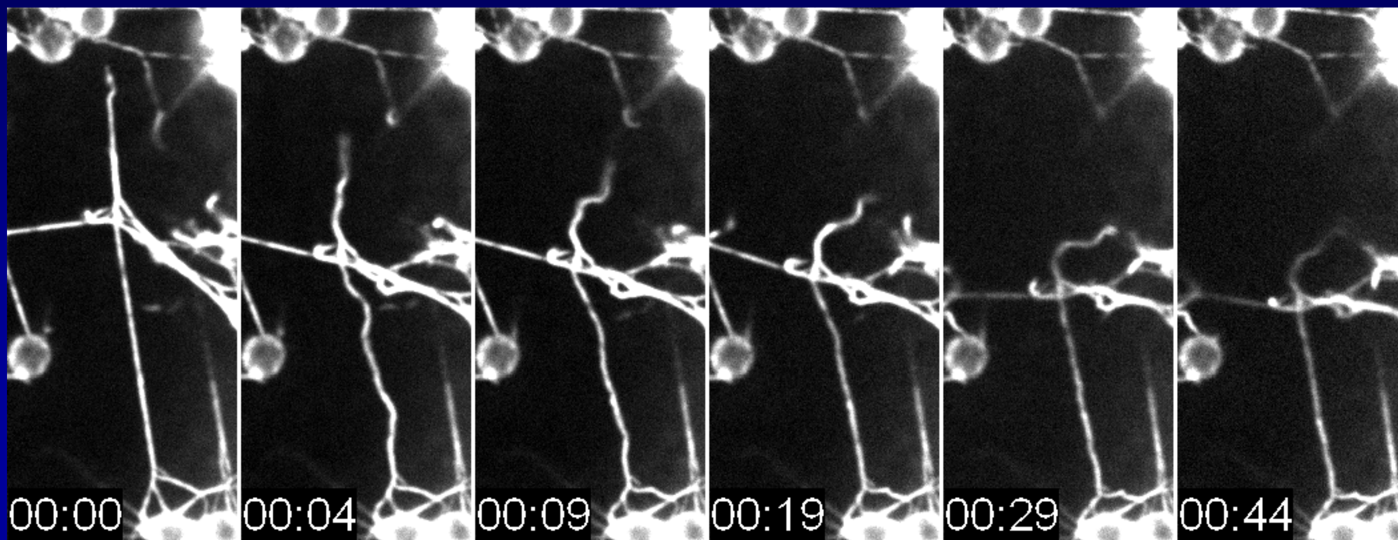
Contractile Remodeling

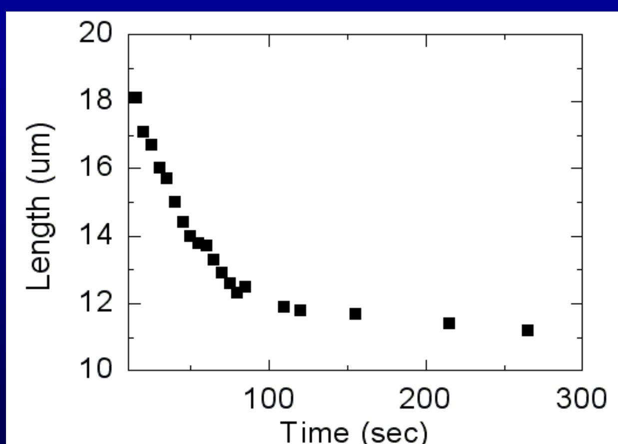
Rupture + Snaps

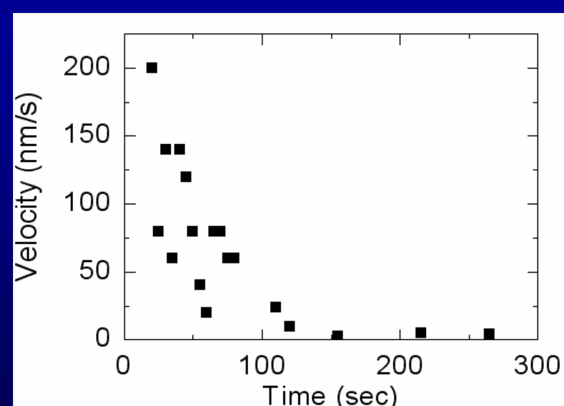


Slack, Stable Bundles

Contractile Remodeling



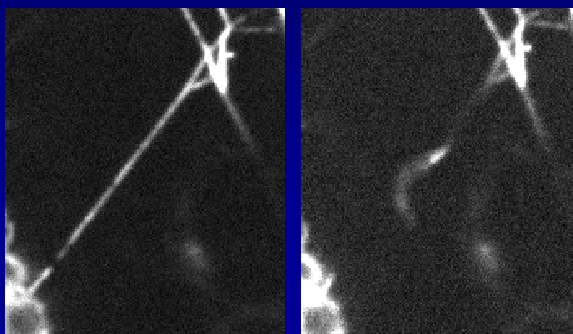




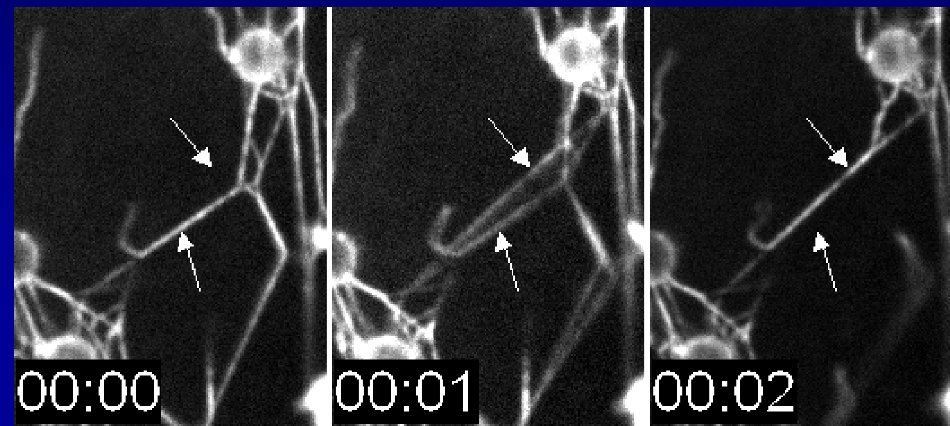
Contracts 30% of length!

Contractile Remodeling

Ruptured snaps

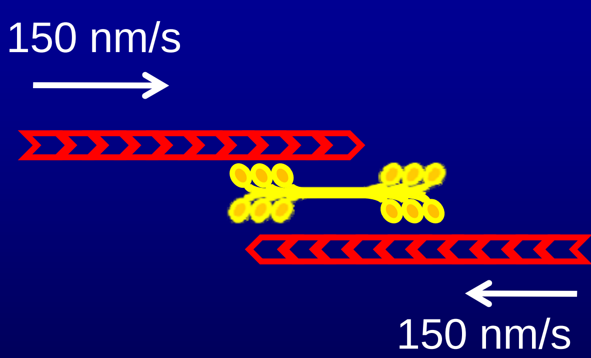


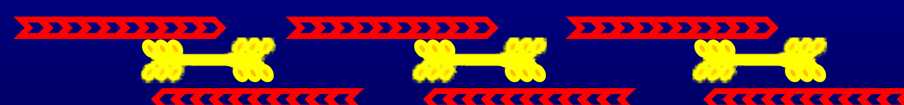
300-400 nm/s



1 μm in <300 ms exposure

> 3000 nm/s



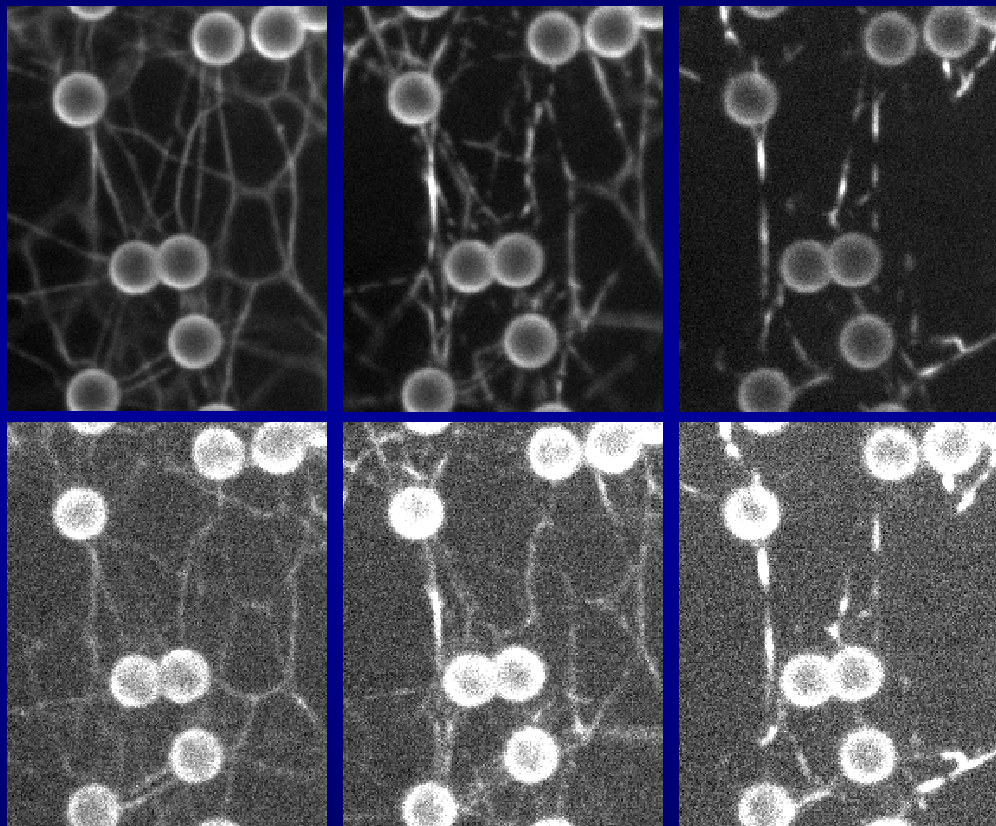


→Tension + Length dependent contraction?

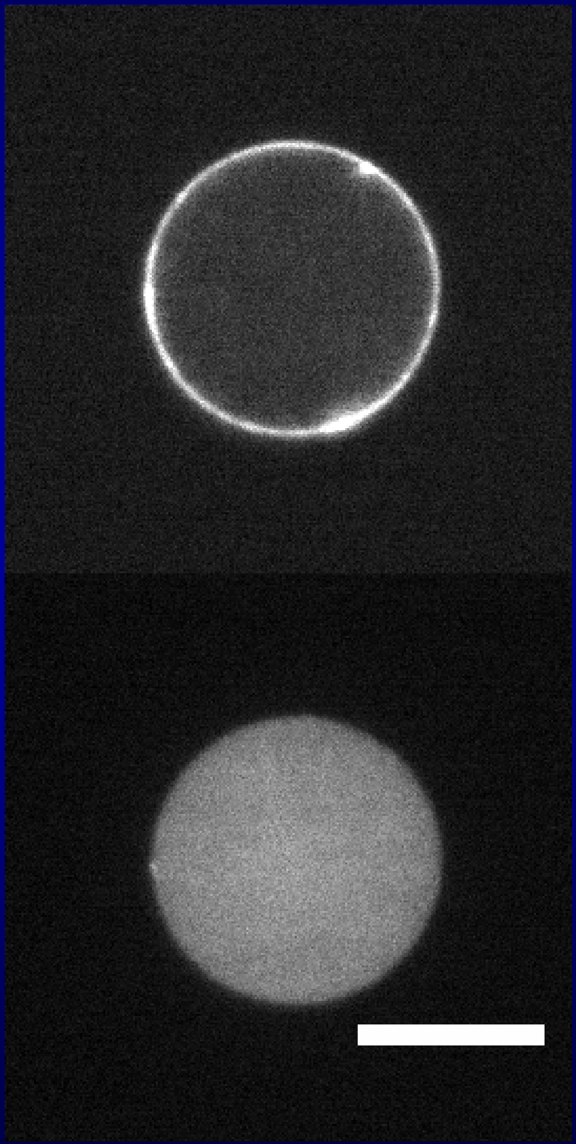
Compaction of F-actin and Myosin During Contraction

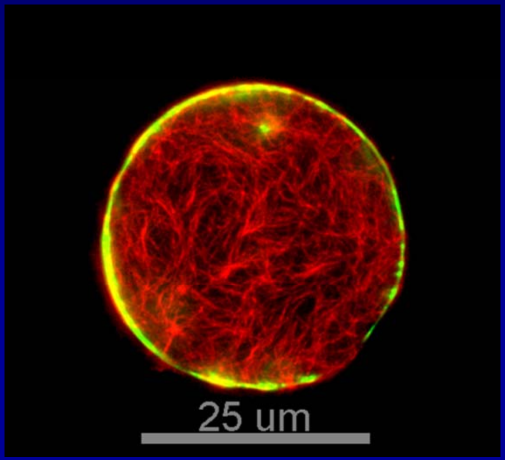
Myosin

F-actin



Model Contractile Cortex





Could we make an in vitro cell Divide?

Questions

•Actin-myosin structures are stable under high tension, rapidly remodel under low tension

•What physical parameters are important for regulating force transmission and ability to remodel dynamically?

•Similarities between microtubule/motors versus actin/motor protein assemblies?

  •Are some extensile?

  •Some contractile?

•Are there similar design principles of cytoskeletal assemblies among diverse sets of proteins that are used to in dynamic biological matter?