

Mechanics of the Lamellar Actomyosin Cytoskeleton

Margaret Gardel

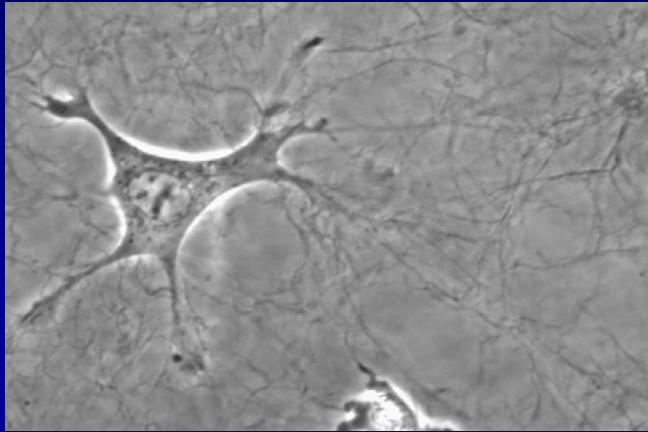
<http://squishycell.uchicago.edu/>

University of Chicago

Physics Department, James Franck Institute, Institute for Biophysical Dynamics

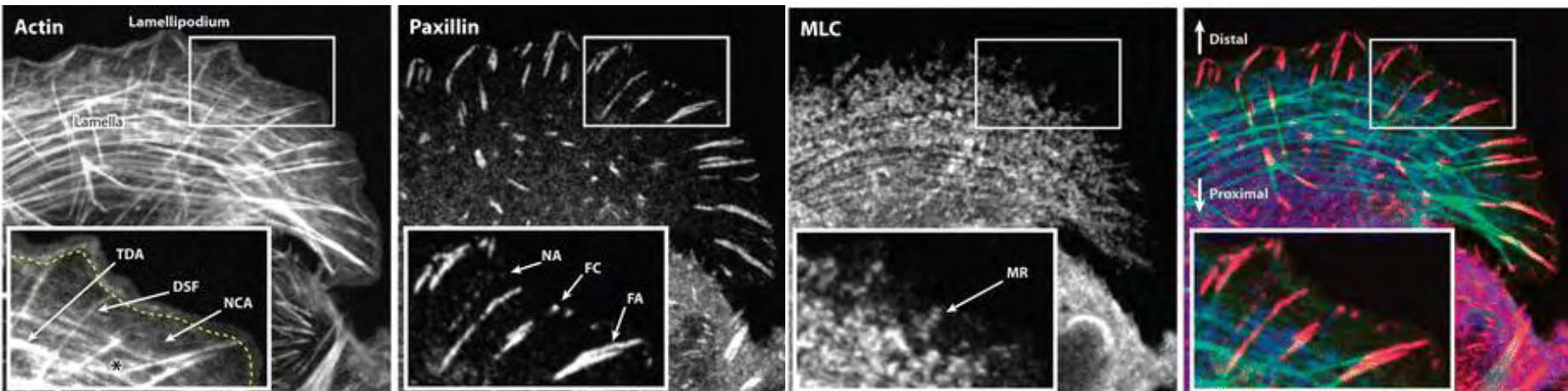
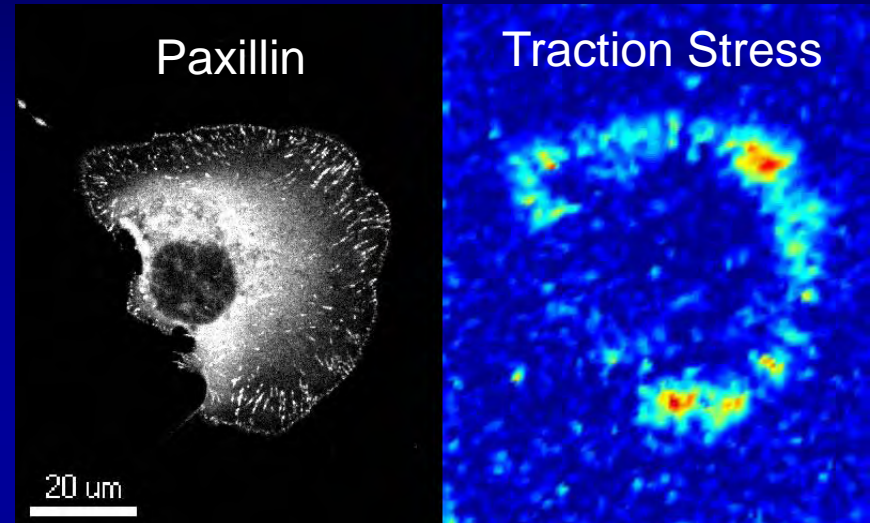
Mechanics of Cell Adhesion and Migration

Endothelial cell in collagen gel

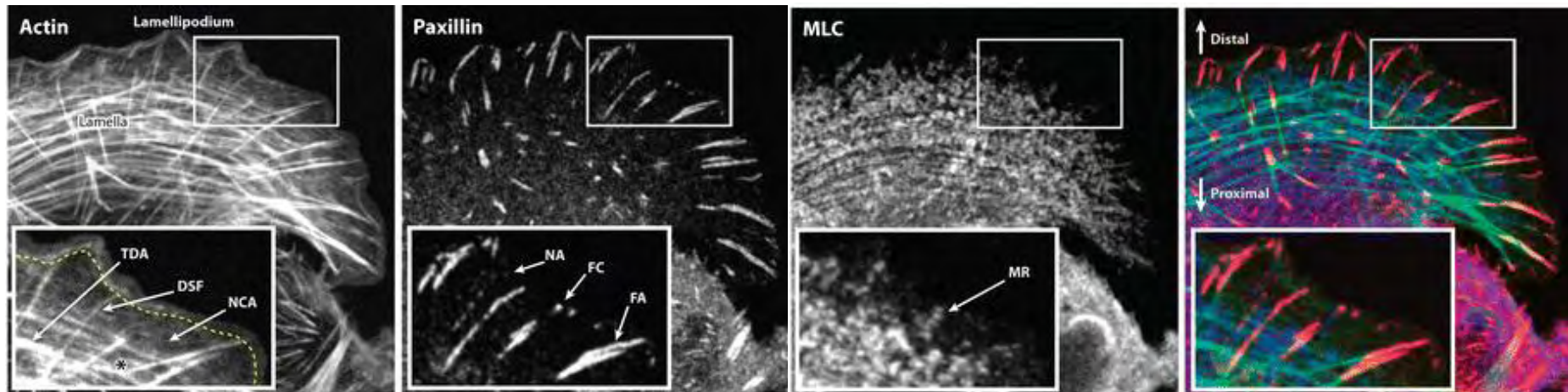
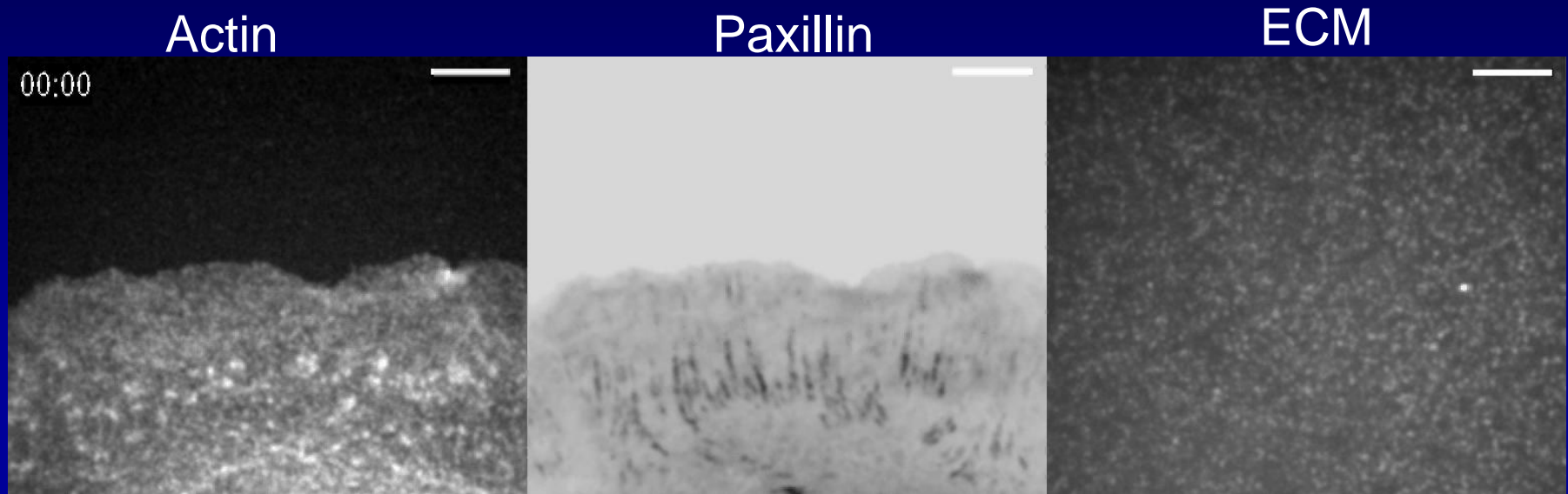


(B. Fischer, NHLBI)

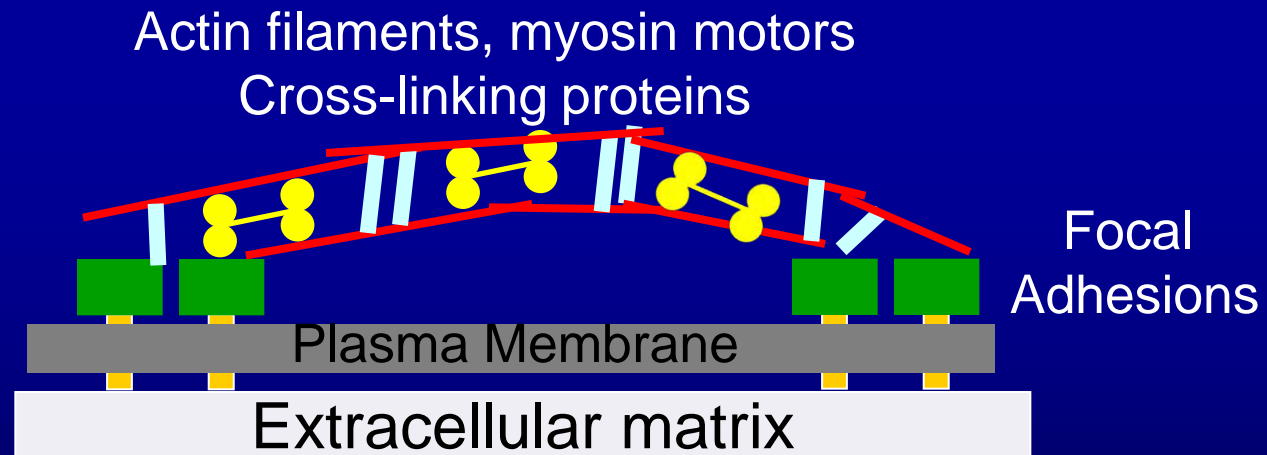
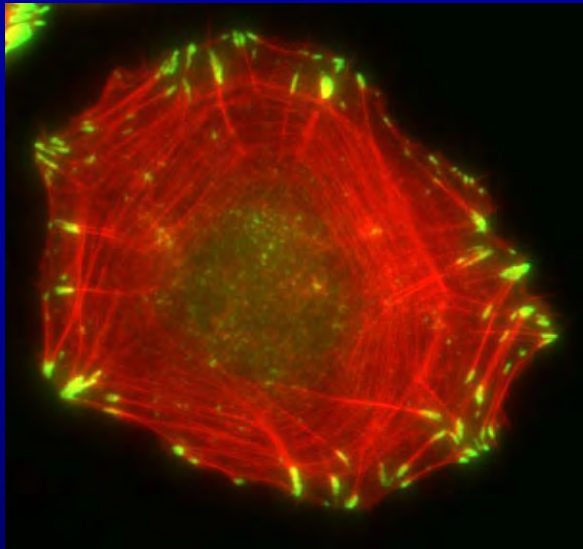
U2OS cell on FN-coated PAA gel



Distinct Actin Structure and Dynamics Drive Migration



Force transmission in Cell Adhesion and Migration

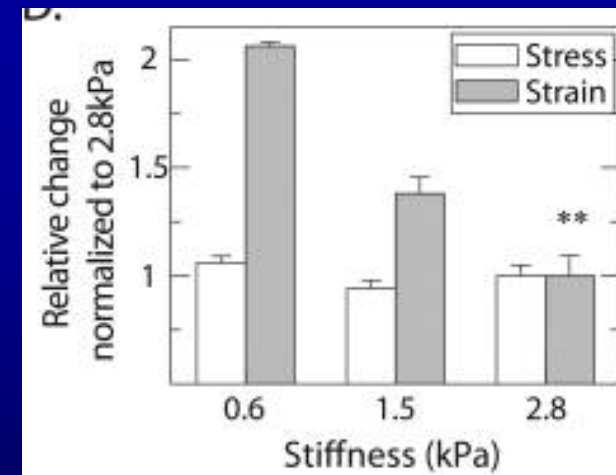
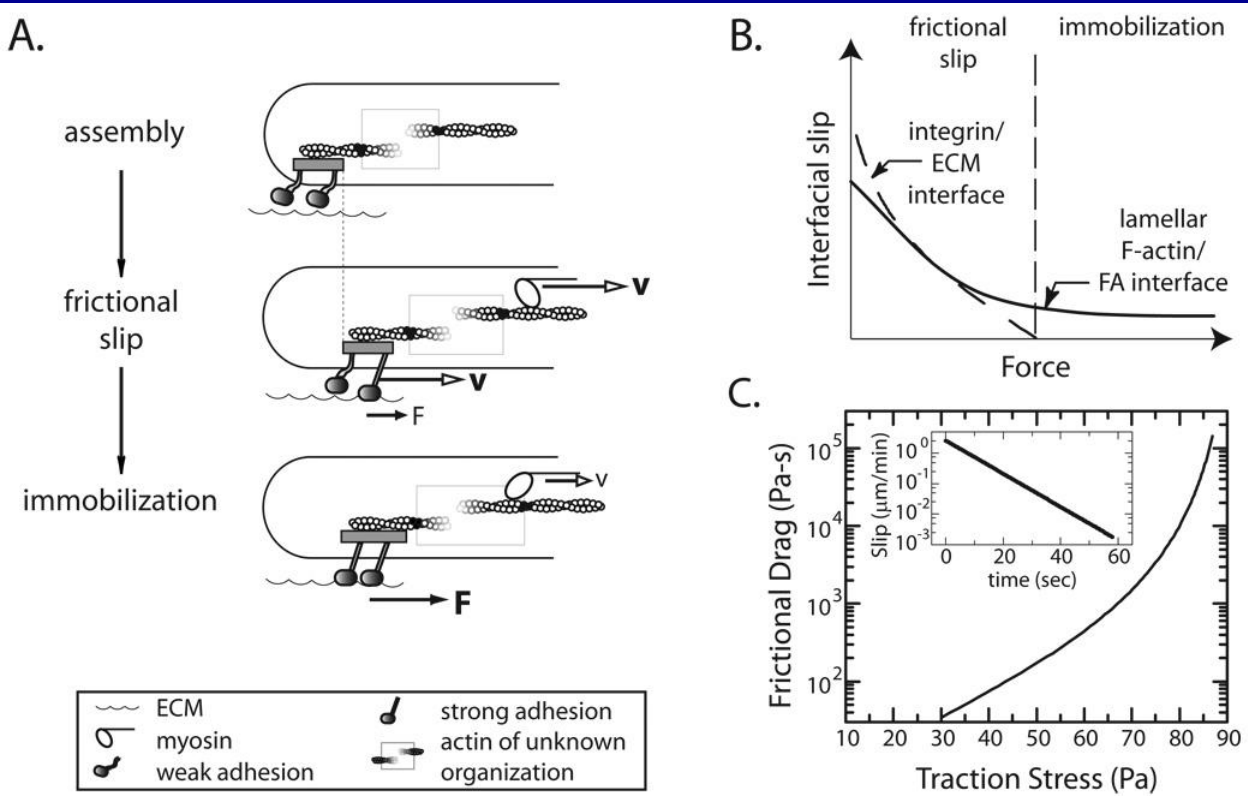


Force transmission in Cell Adhesion and Migration

Actin Cytoskeleton
Force Generation
& Mechanics

Cell-ECM
Adhesion

ECM
mechanics



Force transmission in Cell Adhesion and Migration

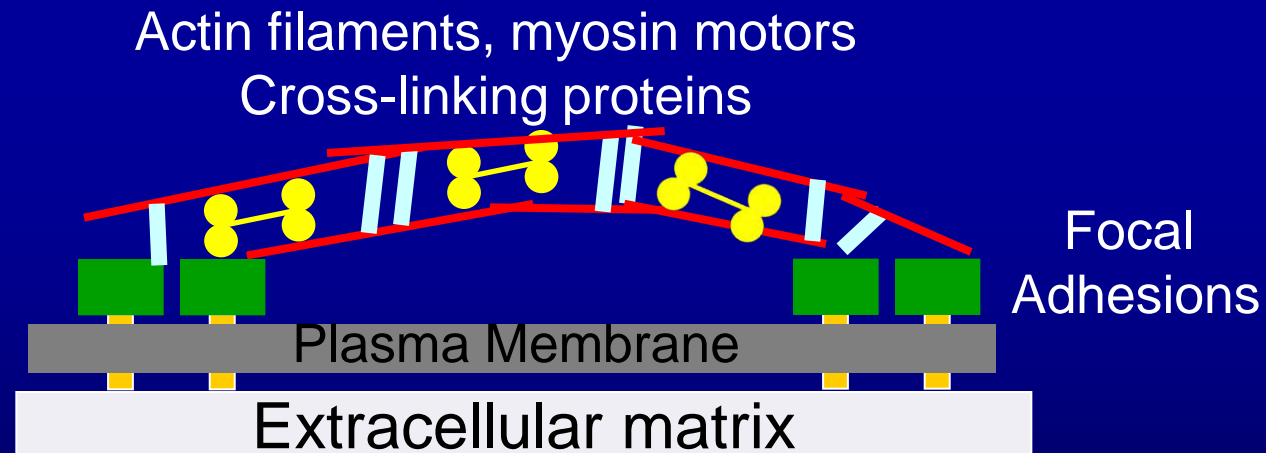
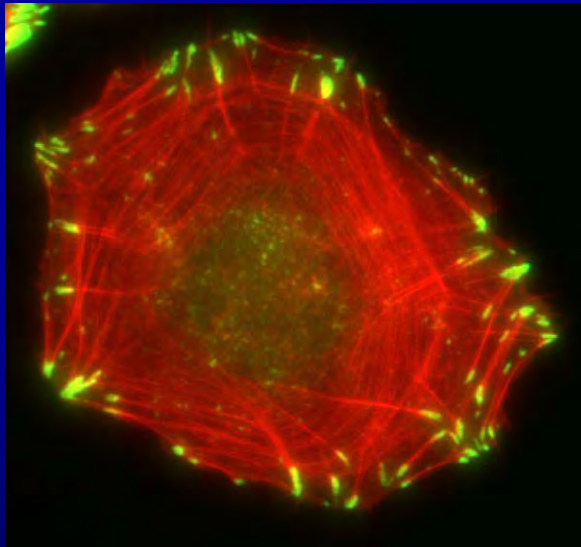
Actin Cytoskeleton
Force Generation
& Mechanics



Cell-ECM
Adhesion

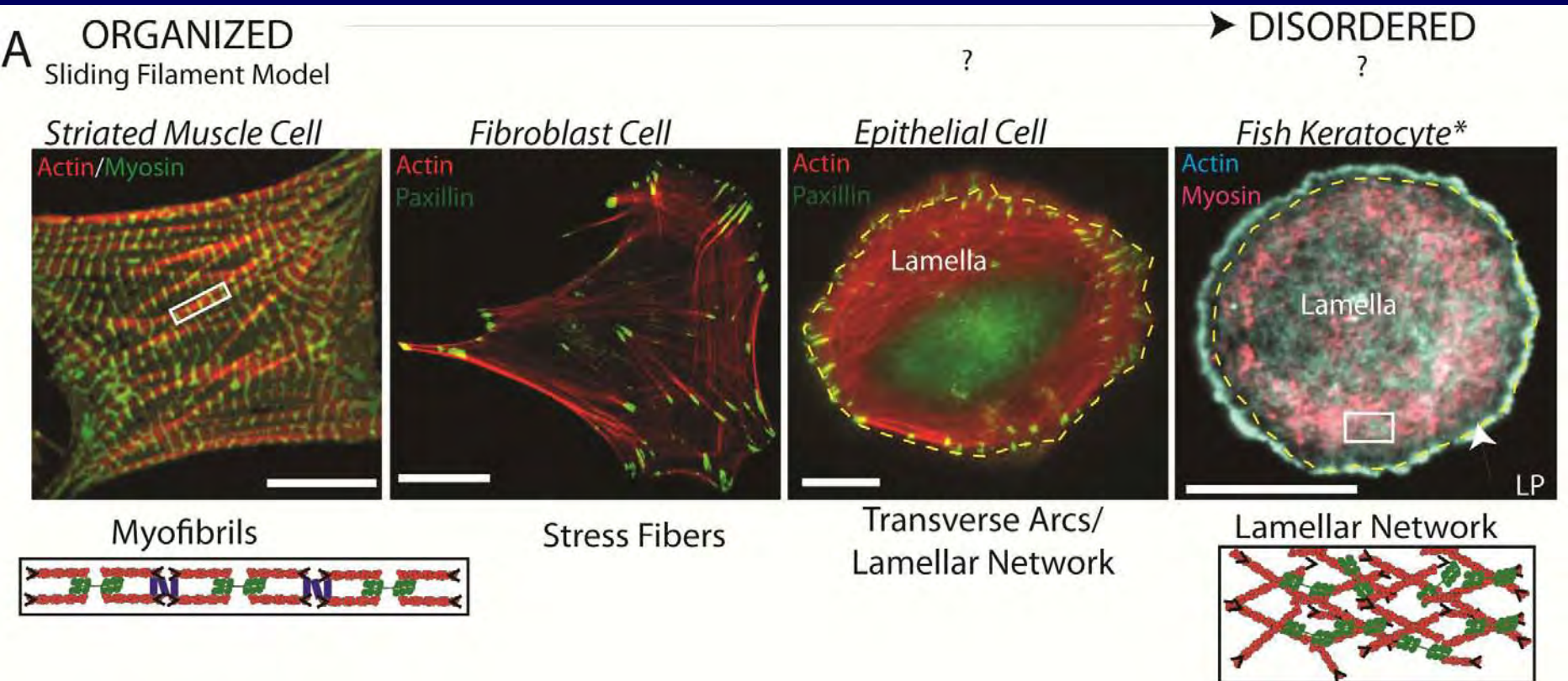


ECM
mechanics



Quantitative predictions of how actomyosin cytoskeleton
transmits forces

Diverse Organizations of Lamellar Actomyosin Cytoskeleton



Non-motile

Stiff ECM (2D)

High Contractility

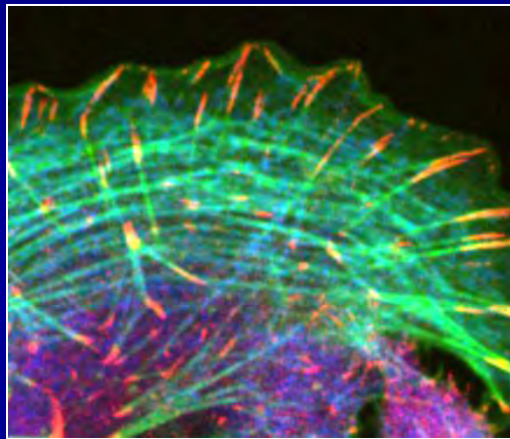
Motile

Soft ECM (3D?)

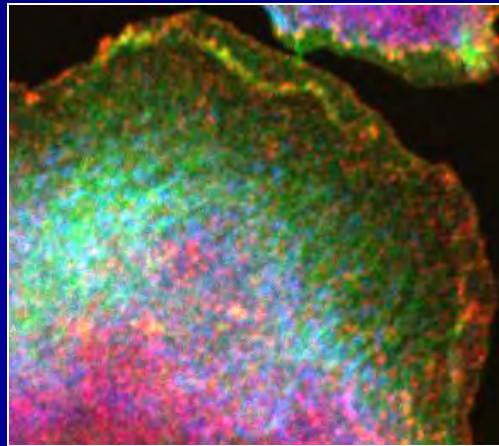
Weak Contractility

Dynamically regulate contractile phenotype

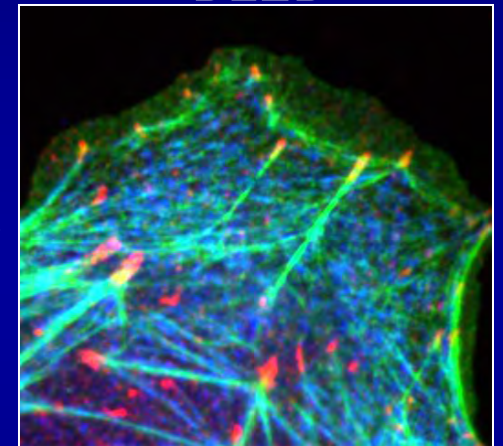
Control



+ 25 μ M Blebbistatin
30 min



15 min after Removing
BLEB



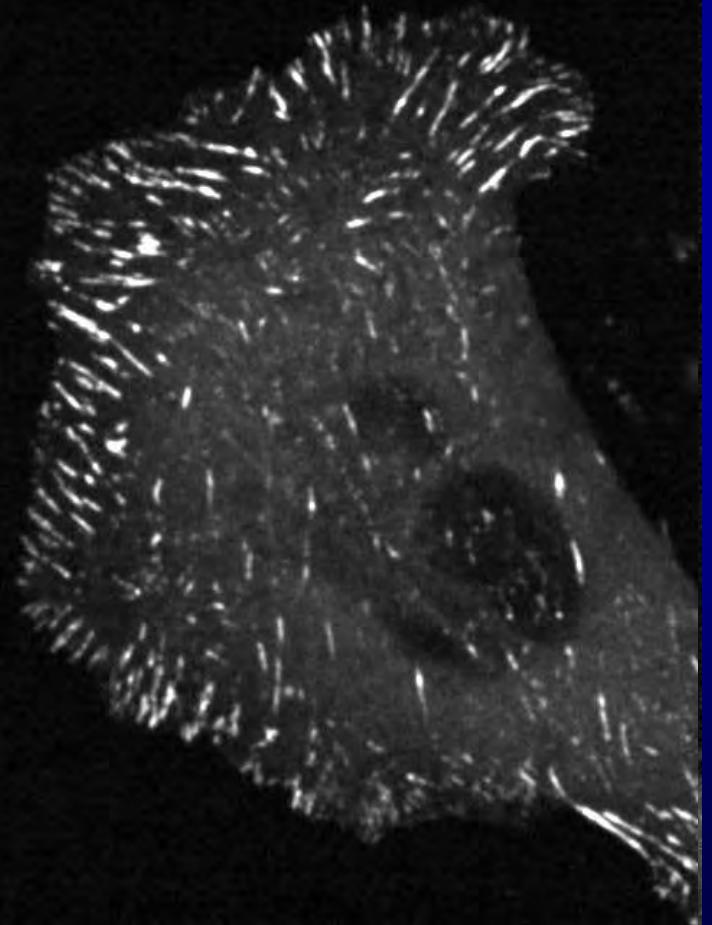
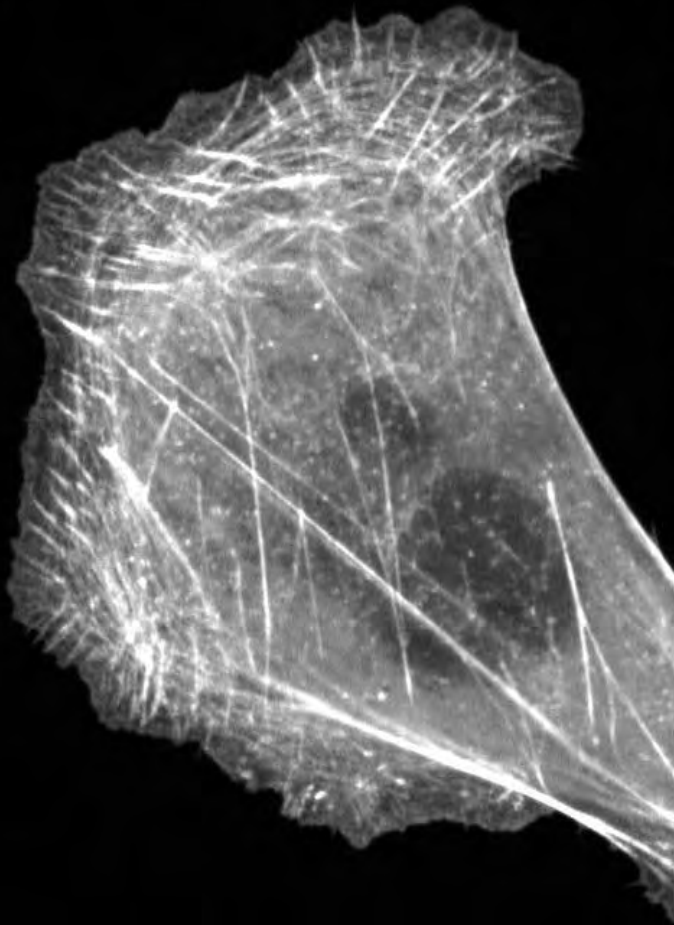
Actin
Paxillin
Myosin Light Chain

Blebbistatin Washout Drives Self-Assembly of Lamellar Networks and Bundles

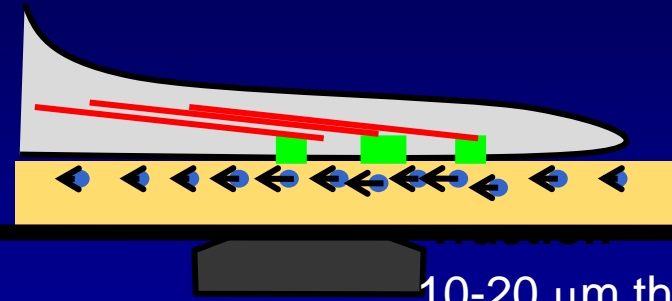
GFP-actin

mApple-Paxillin

10 μm

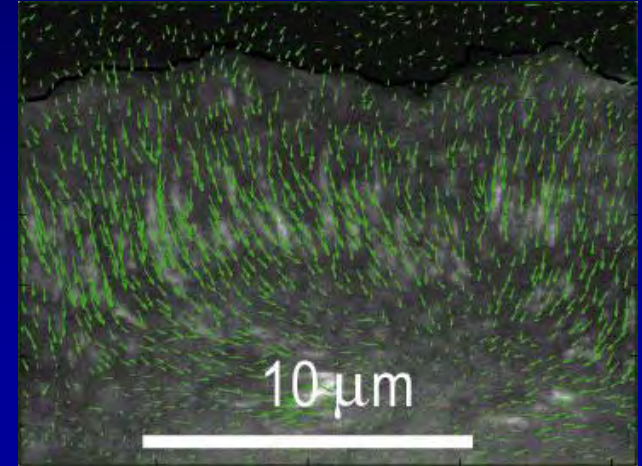


High Resolution Traction Force Microscopy

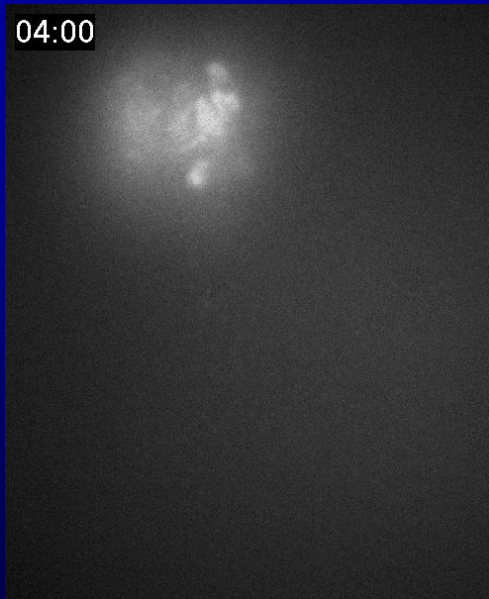


10-20 μm thick polyacrylamide gel
40 nm far red latex spheres

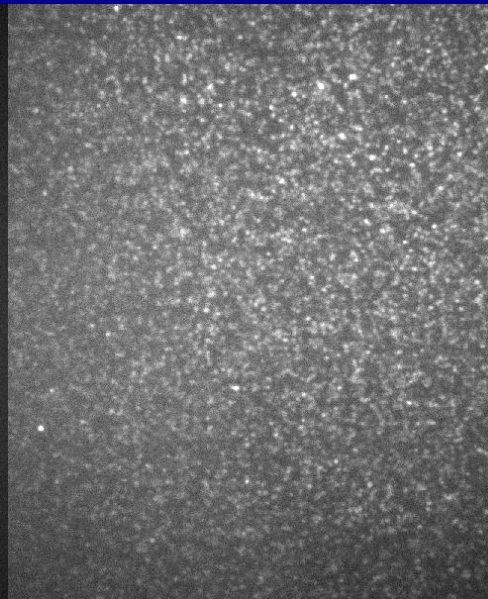
Displacement Field, U



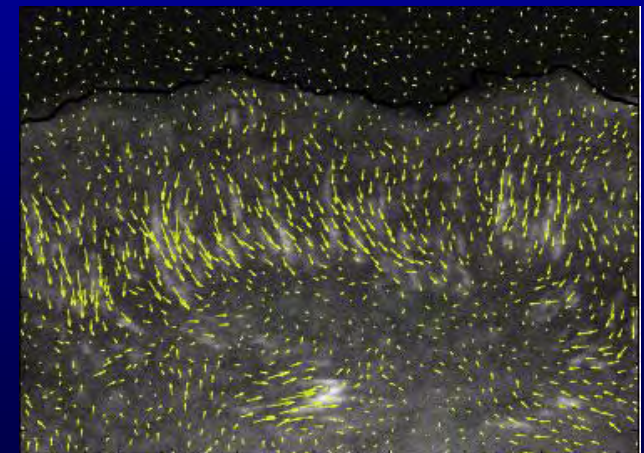
GFP-actin



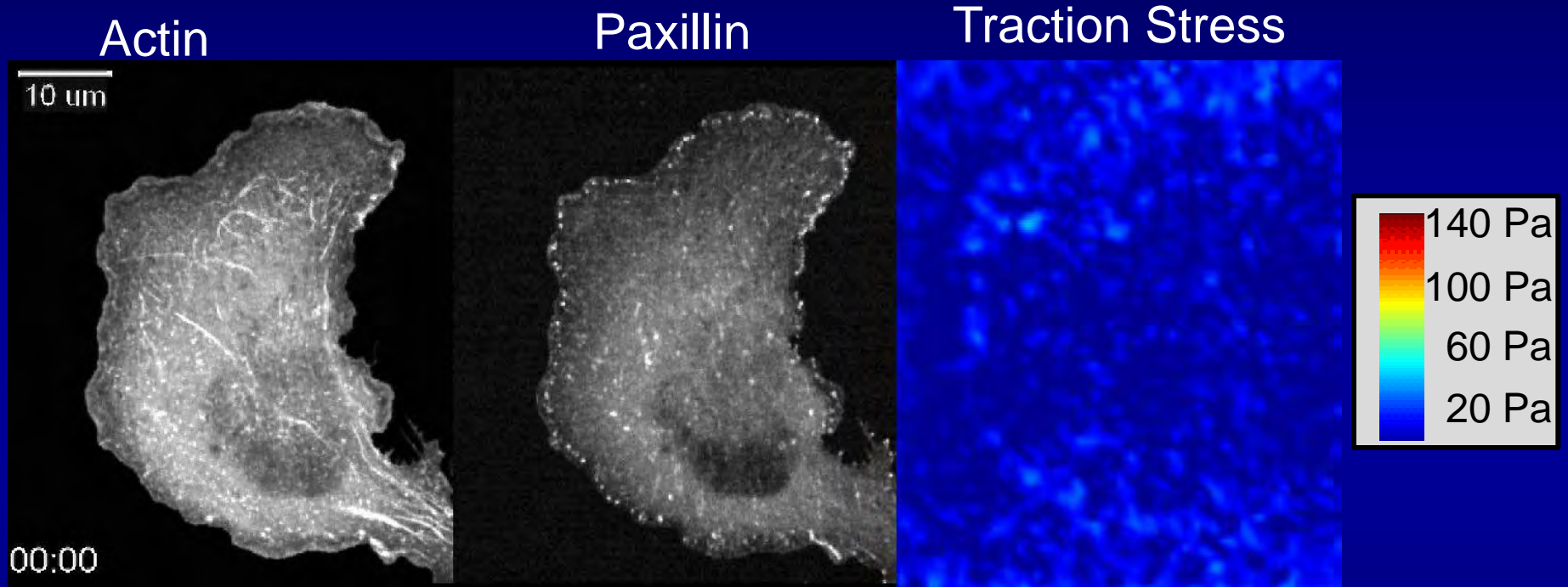
Cy5 beads



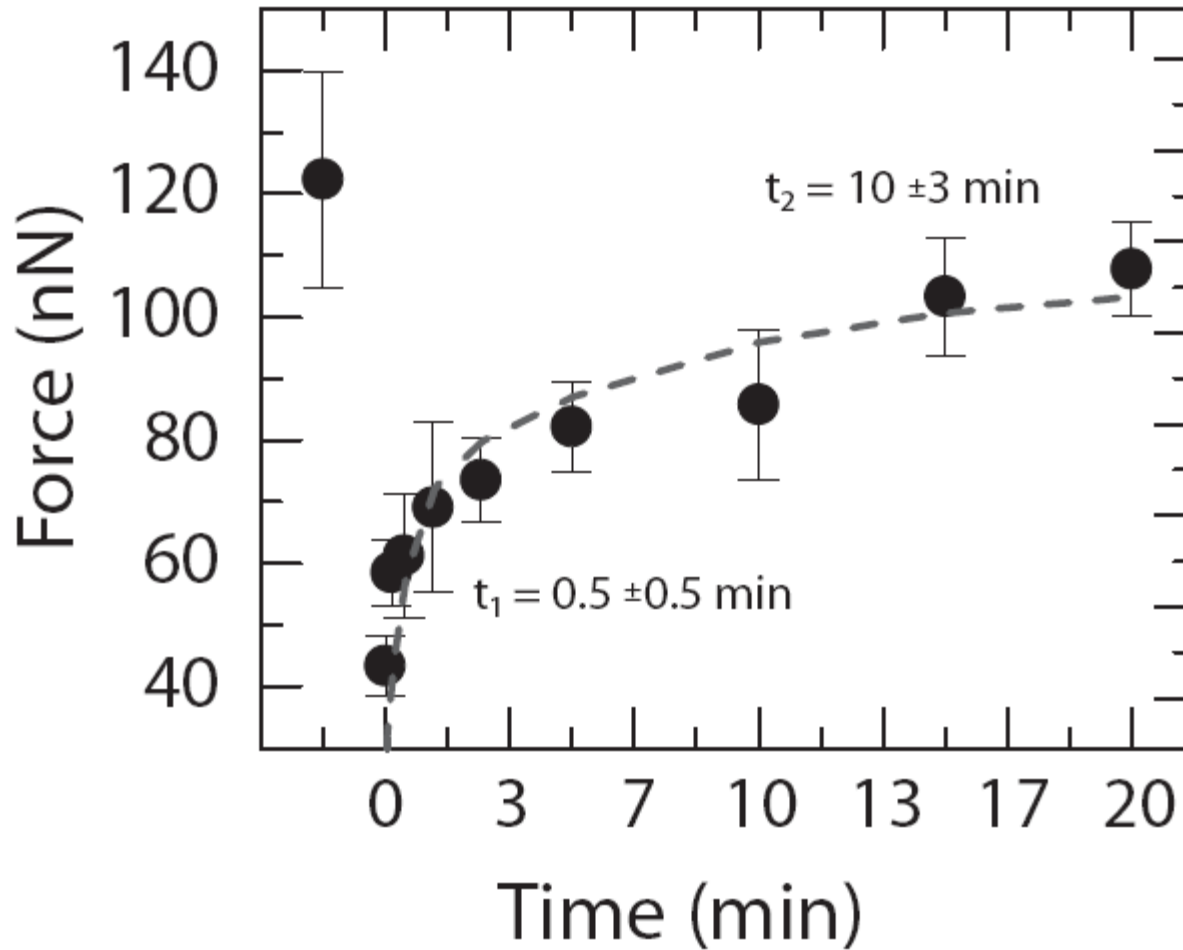
Traction Stress Field, F



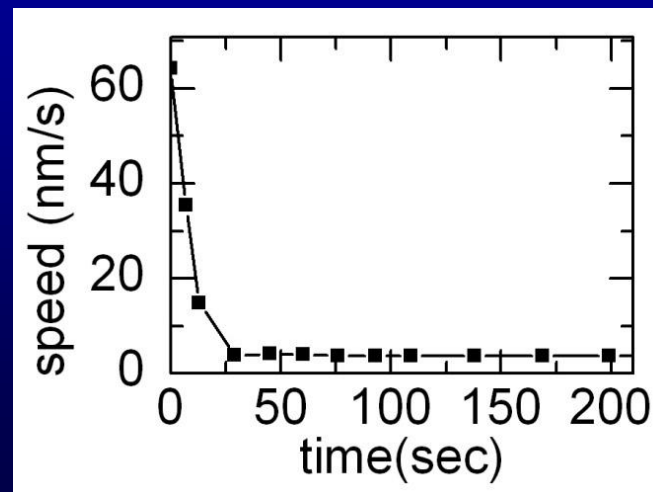
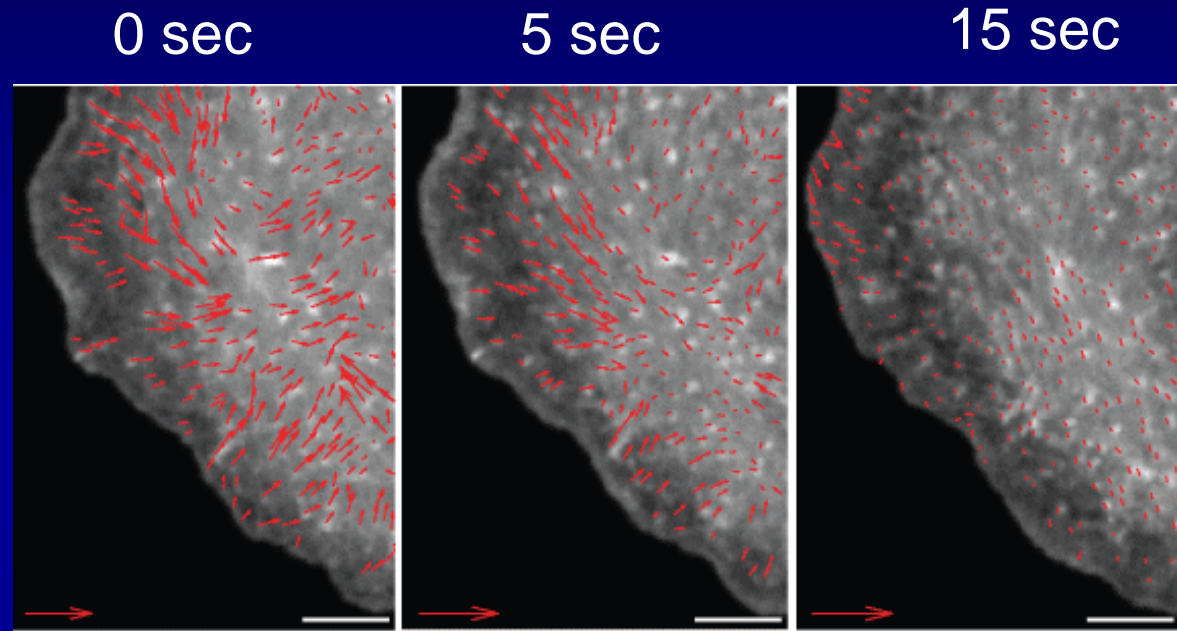
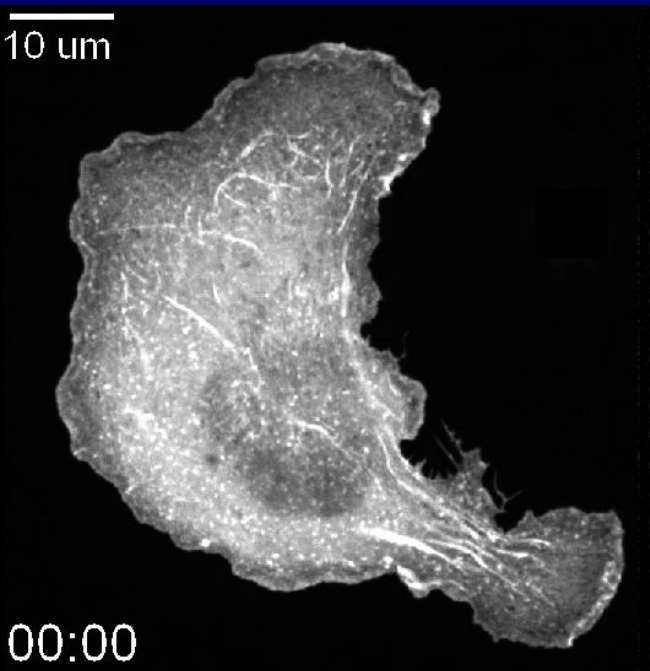
Assembly of Contractile Lamella and Traction Forces



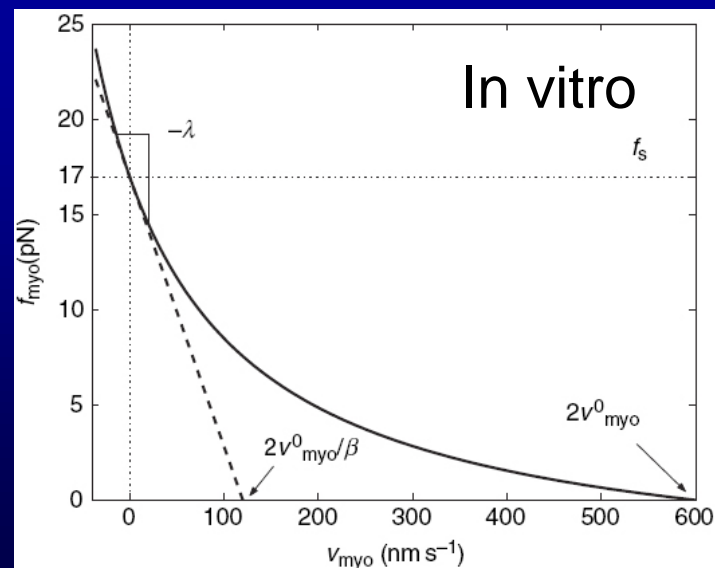
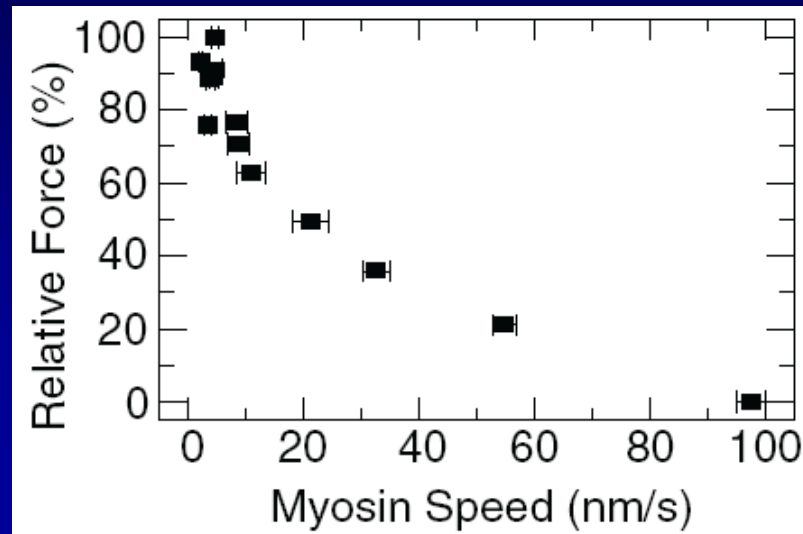
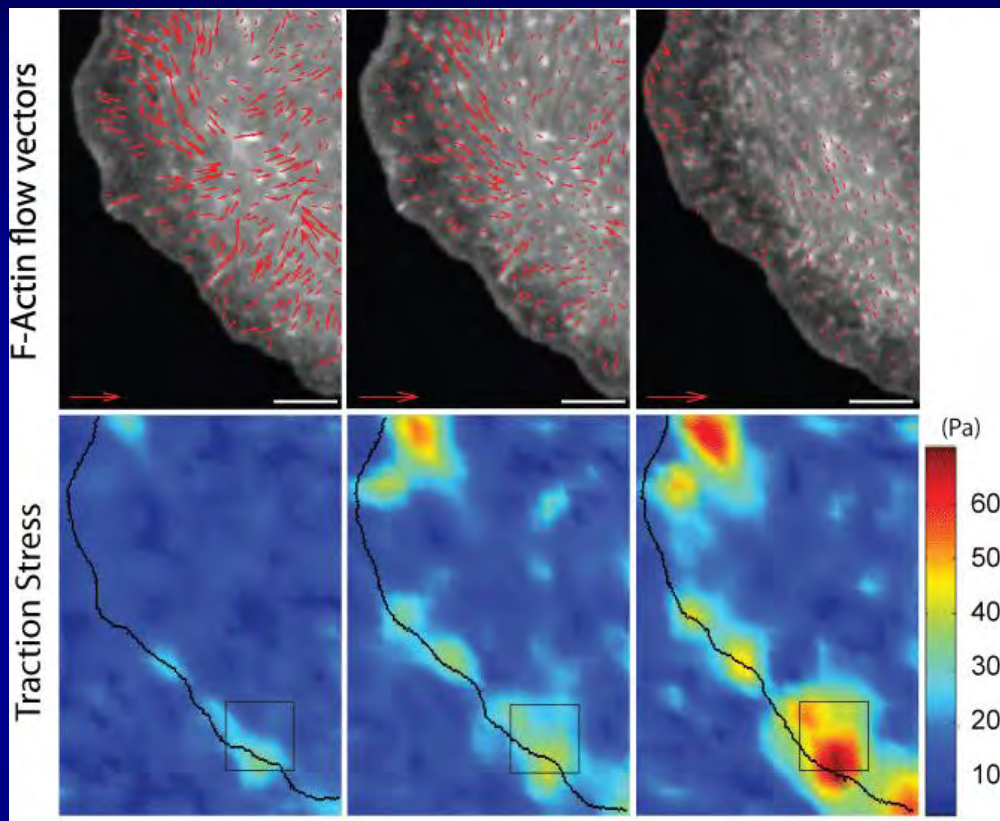
Rapid and Slow Phases of Tension Build-Up



Fast Time Scale: Rapid Actin Dynamics

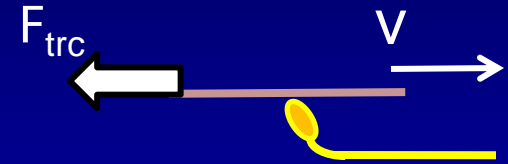


Inverse Force-Velocity Relationship for Lamellar Actin Networks

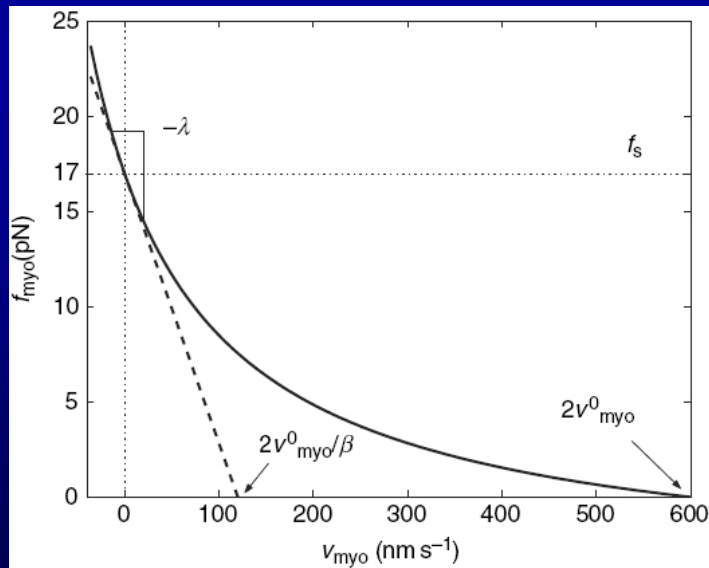


Lamellar mechanics mimics myosin II mechanochemistry

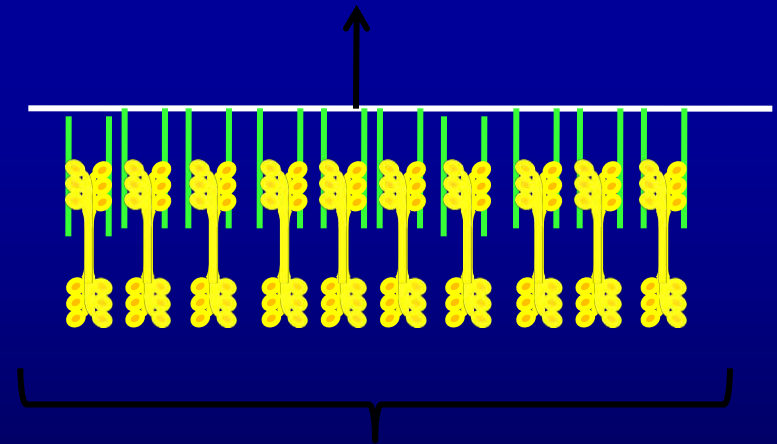
	Cell	<i>In Vitro</i>
v_u	108 nm/s	100 nm/s
F_{stall}	200 nN	~5 pN



In vitro



$F = 200 \text{ nN}$

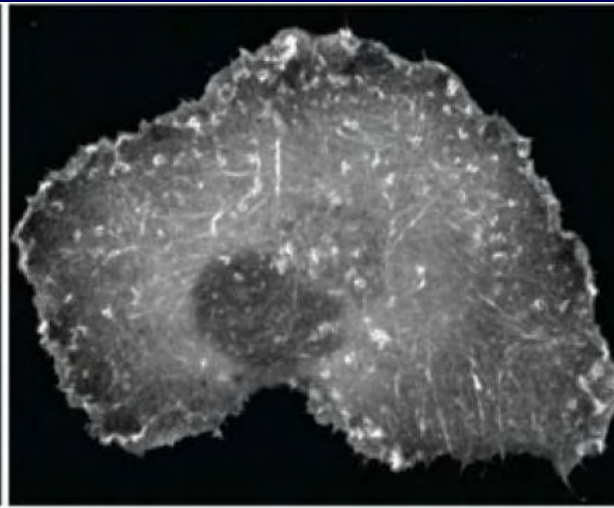
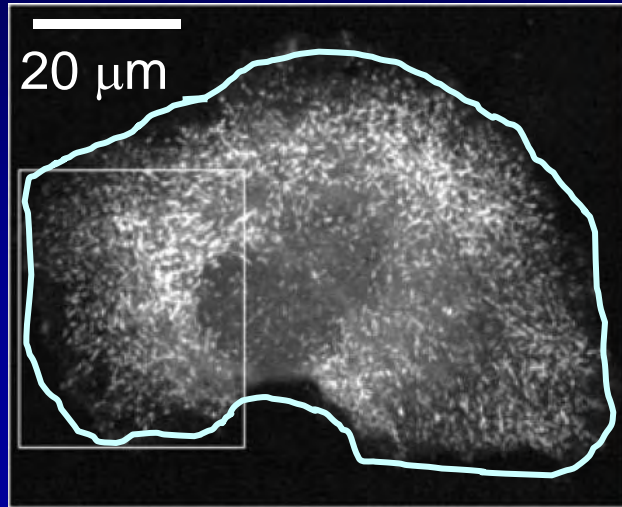


40000 motors working in parallel

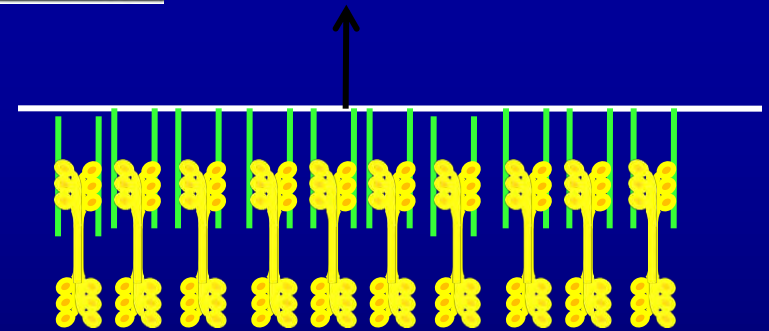
Lamellar mechanics mimics myosin II mechanochemistry

GFP-Myosin

mApple-Actin



$F = 200 \text{ nN}$



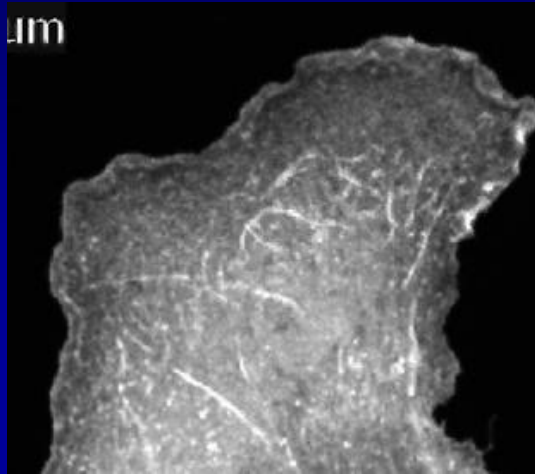
$0.5 \mu\text{m}$

$400 \mu\text{m}$

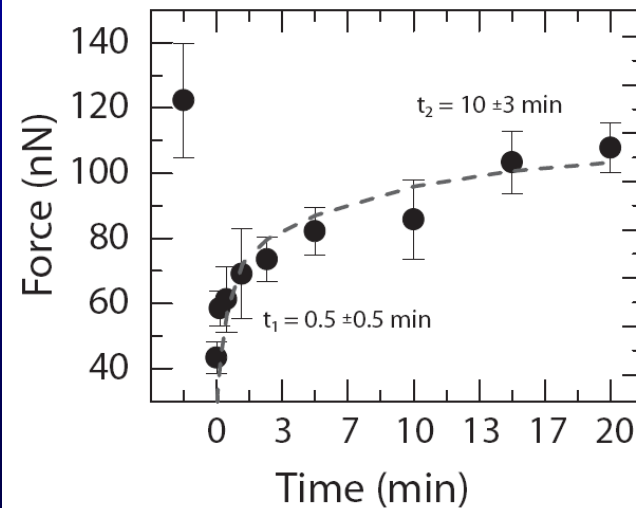
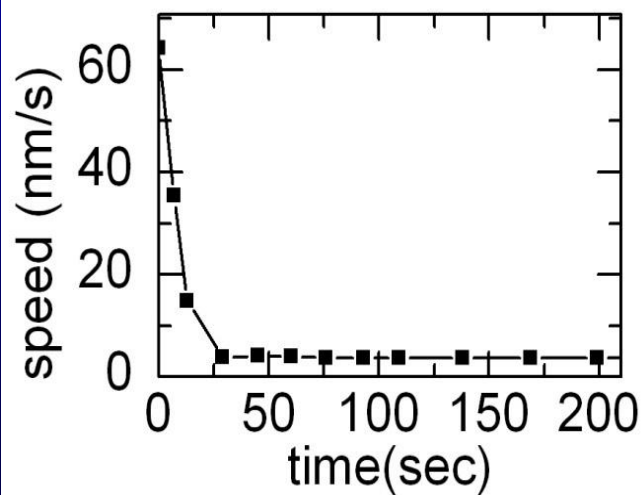
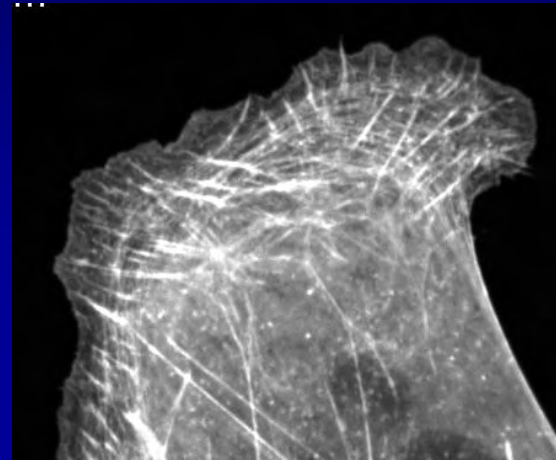
- $\sim 500 \text{ pN}/\mu\text{m}$
- $\sim 5 \text{ pN}$ stall force/motor
- ~ 100 motors/ μm working in parallel
- ~ 2 puncta/ μm
- 8 motors per minifilament
- 6 minifilaments per puncta

Long time scale: Stress Fiber Formation

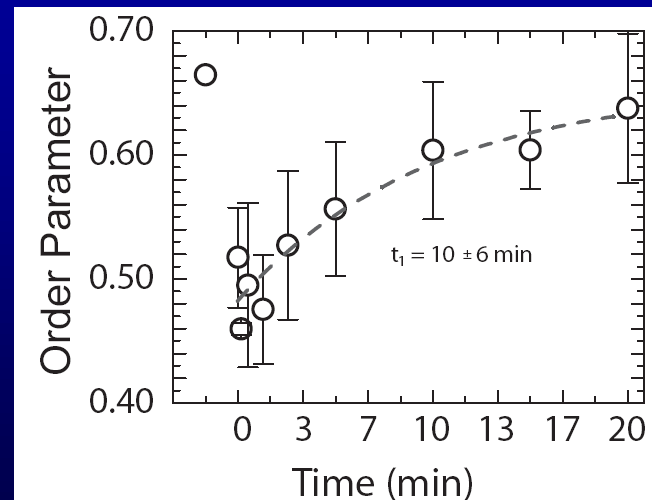
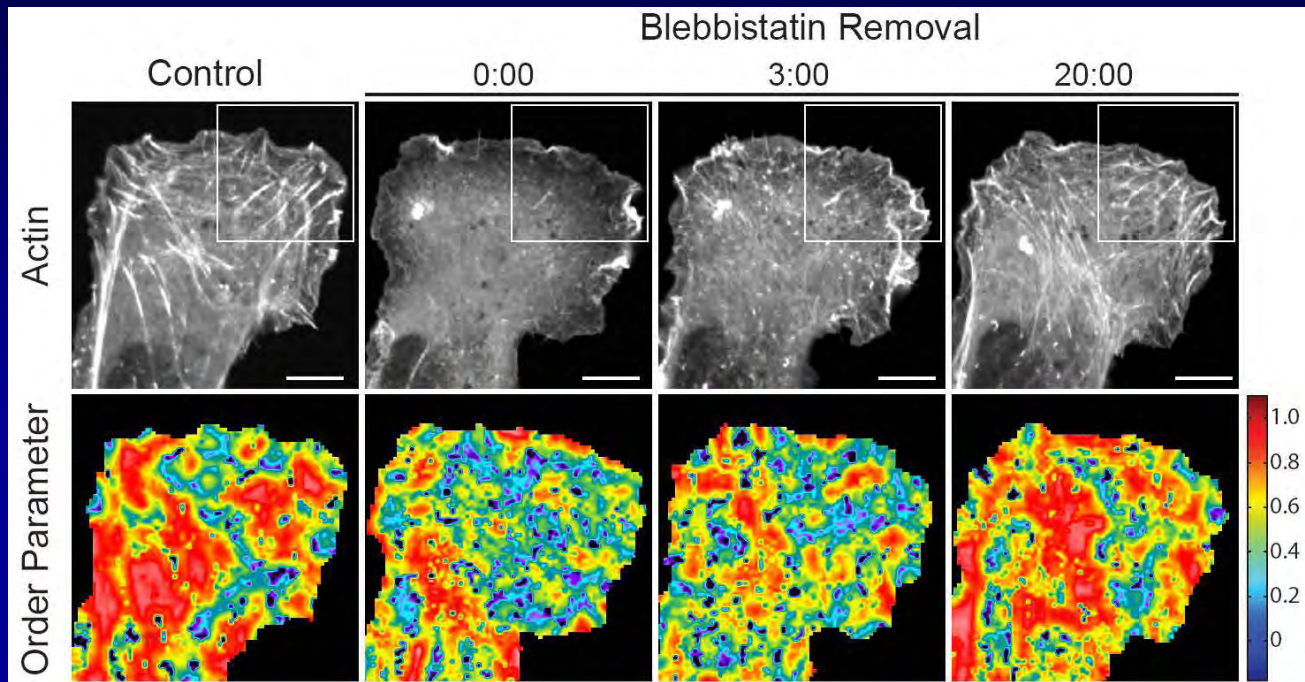
00:30



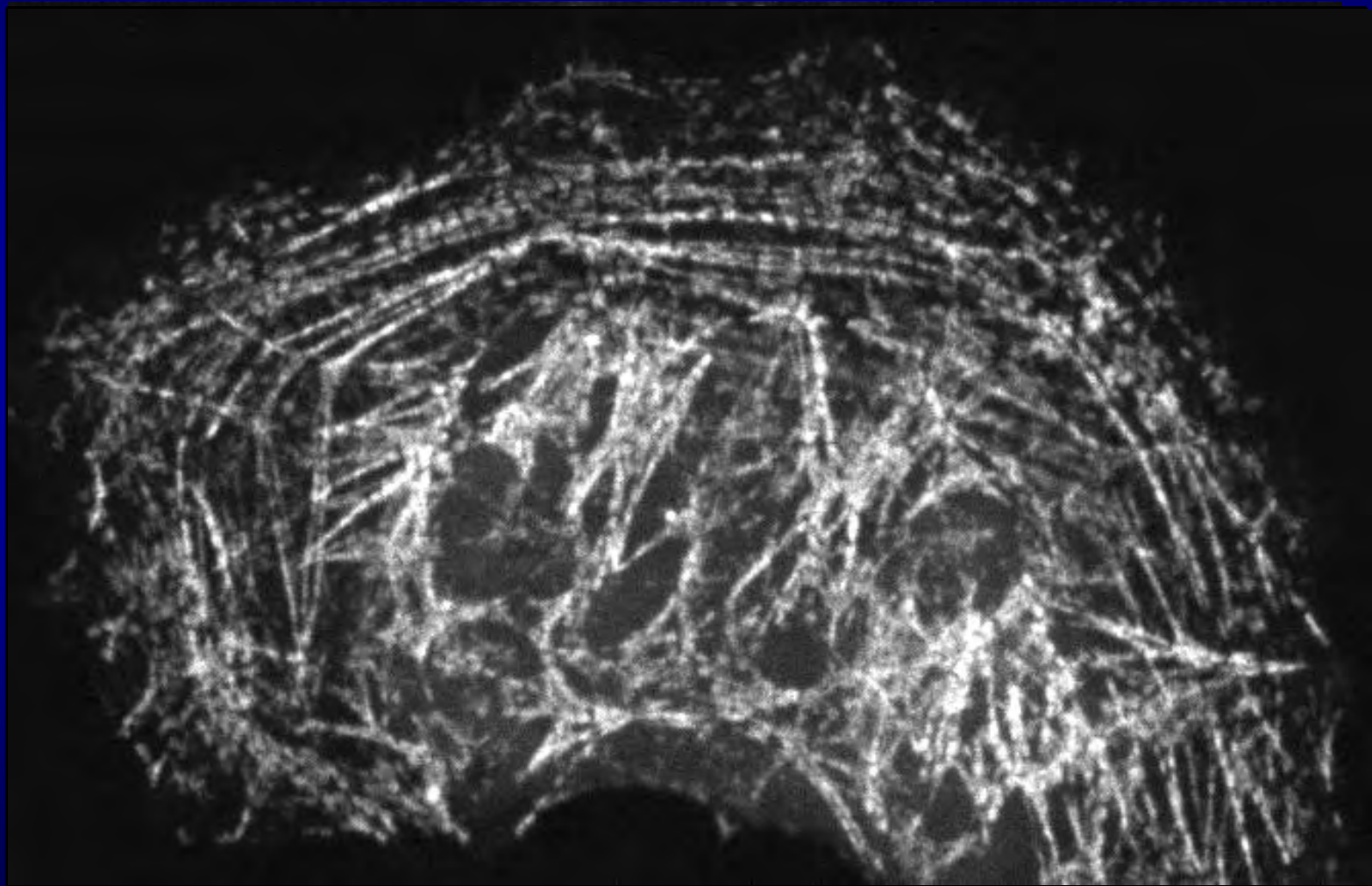
30:00



Order Parameter to Measure Extent of Bundling

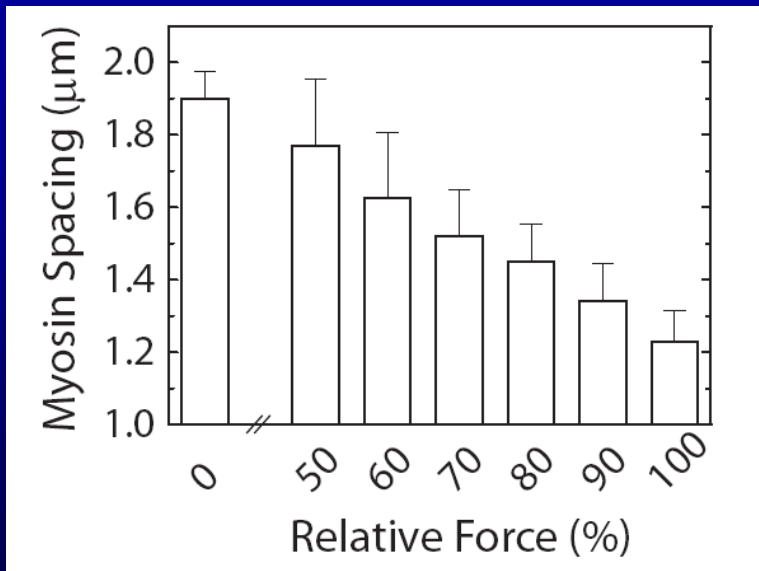
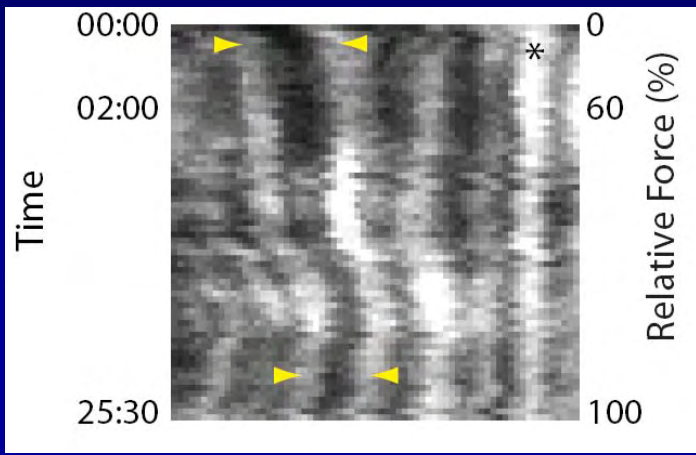
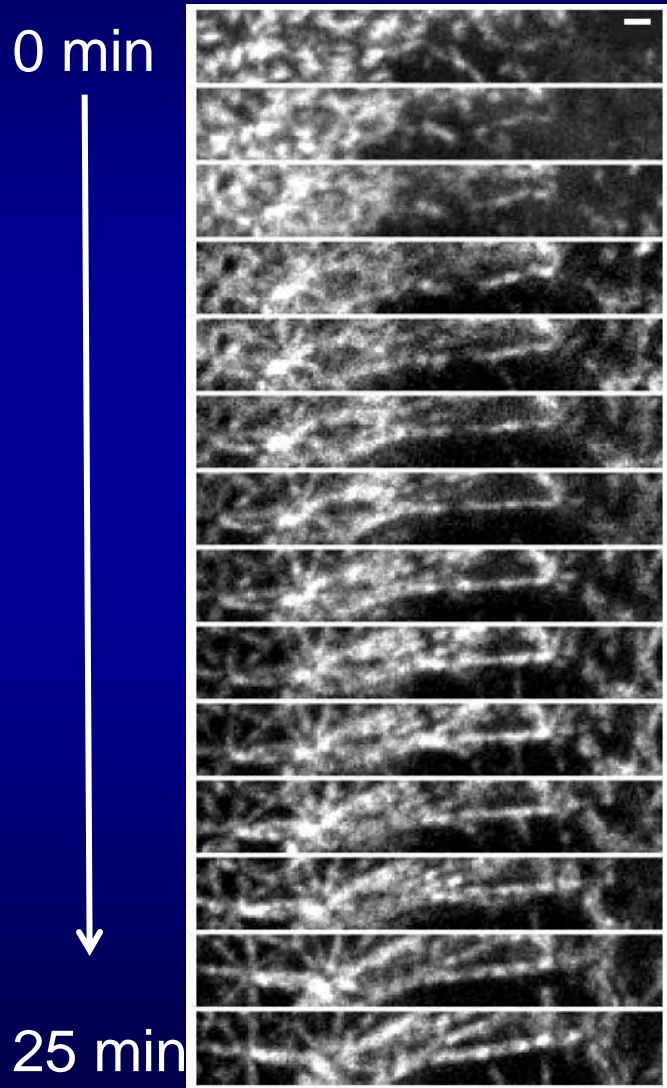


Myosin II drives stress fiber formation



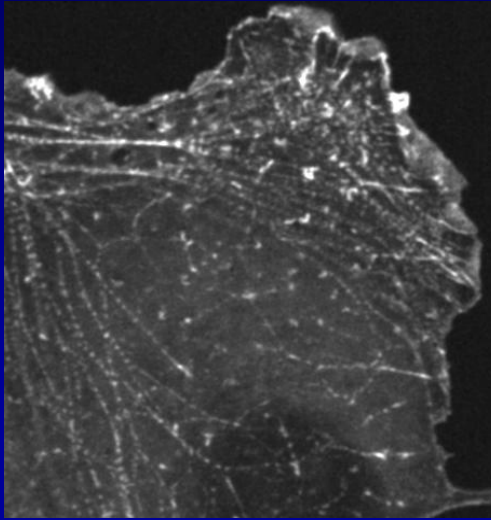
GFP-myosin light chain

Myosin band spacing decreases as tension builds

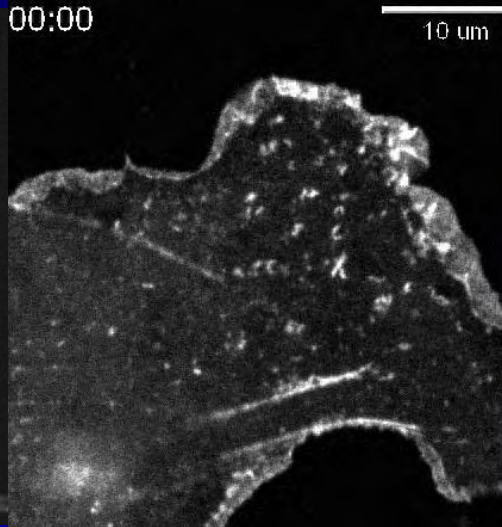


α -actinin bands form at increased tension

Control



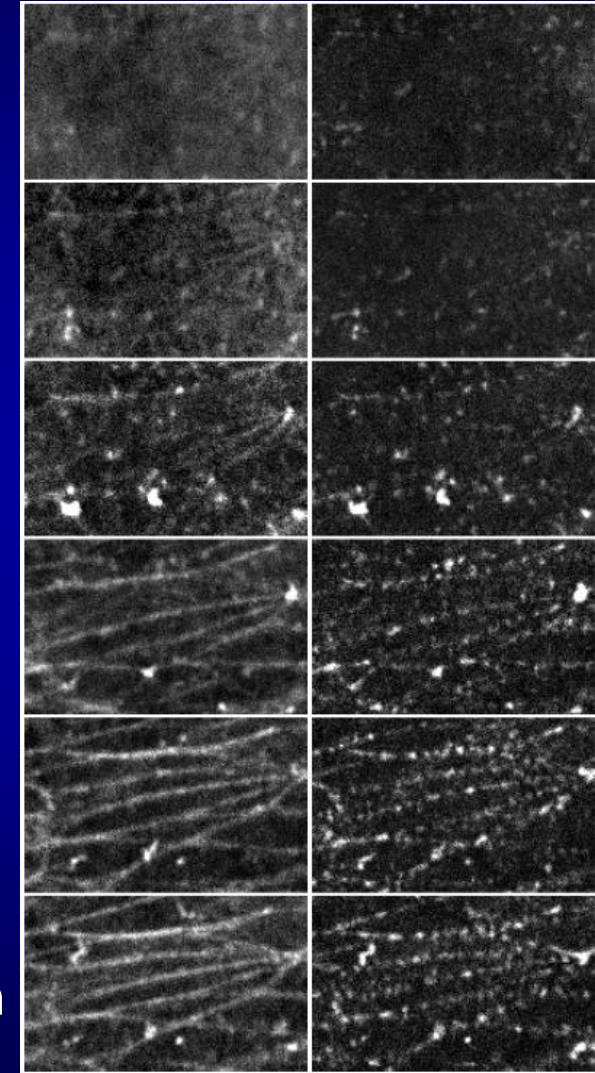
Bleb. Washout



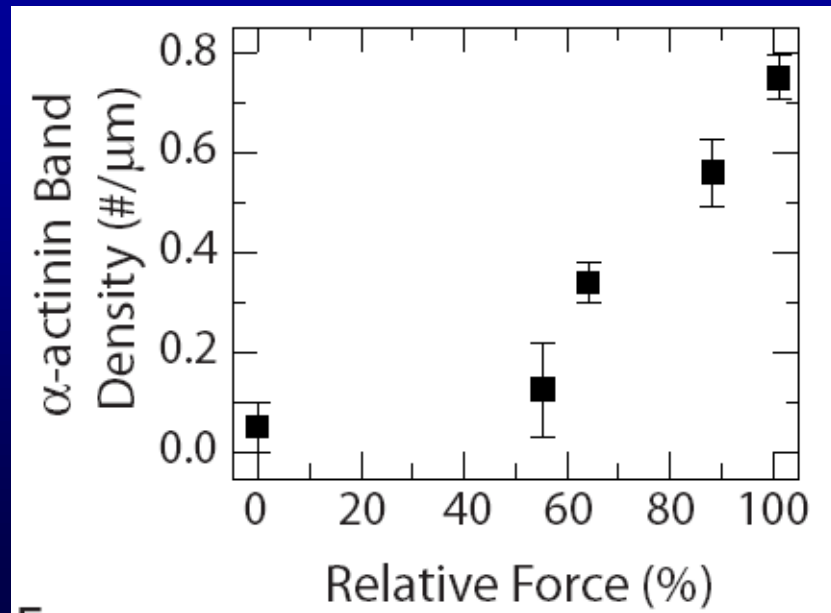
Actin

α -actinin

0 min



35 min



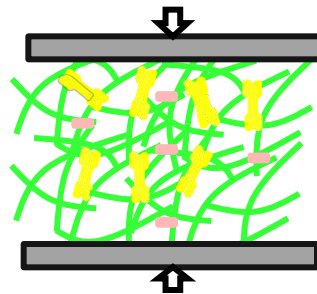
Lamellar Architecture regulates Force Transmission

Myosin-Driven Tension

0% ————— 50% ————— 70% ————— 100%

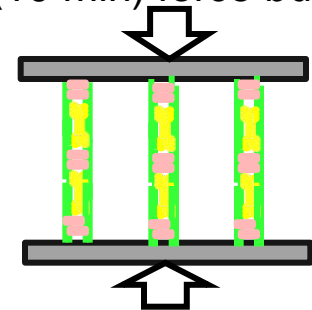
Actin Organization	Lamellar Network	Stress Fibers form + thicken
<i>Myosin bands</i>	random	~2 μm —————> ~1 μm
α -actinin bands	N/A	form + intensify ~1.5 μm —————> ~1 μm
Dynamics	25 nm/sec —————> 5 nm/sec	5 nm/sec

Dynamics dictate Force
Rapid (30 s) force build up



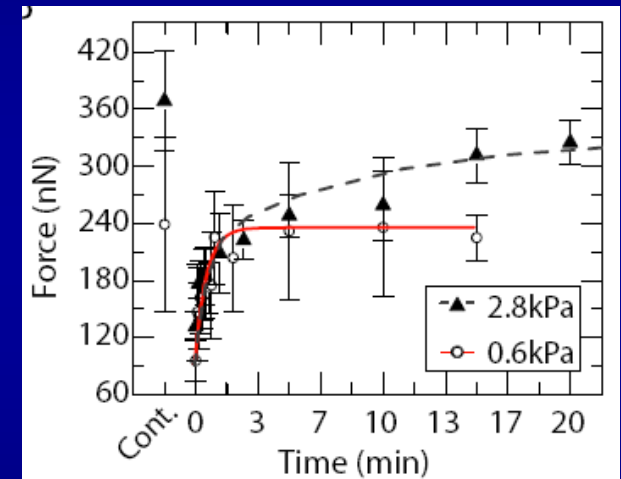
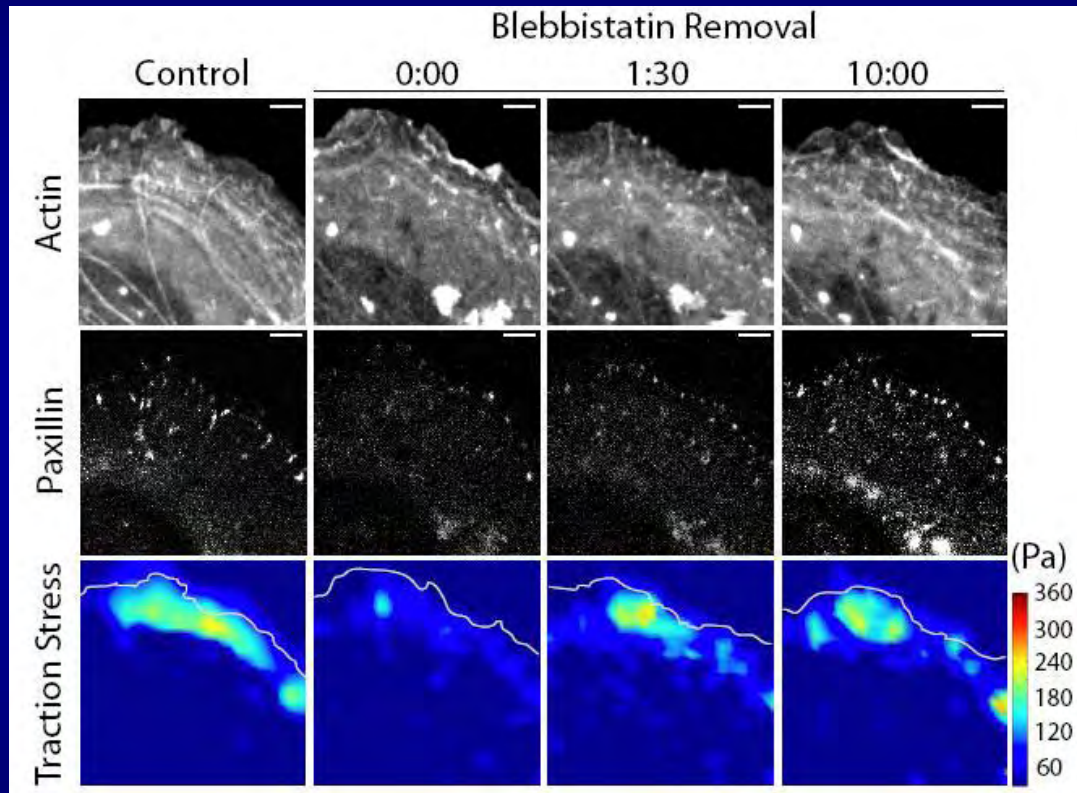
“Active” Fluid
(Mogilner, J.F. Joanny, K. Kruse)

Structure dictates Force
Slow (10 min) force build up

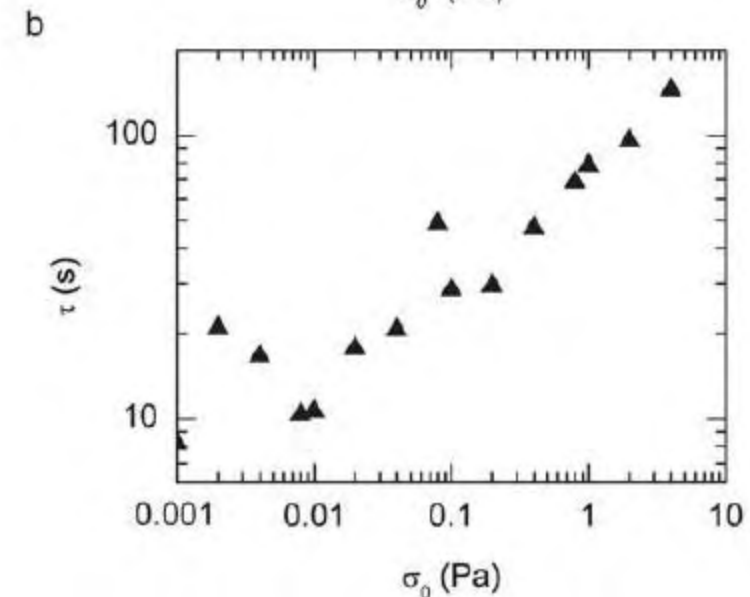
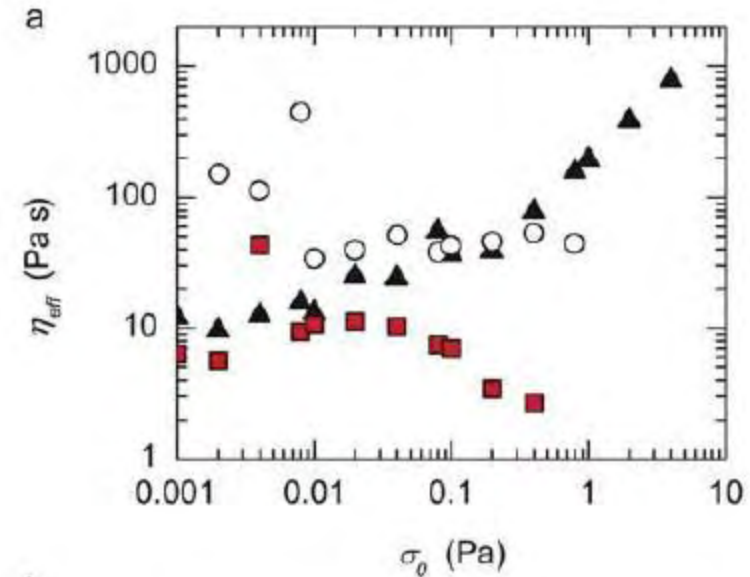
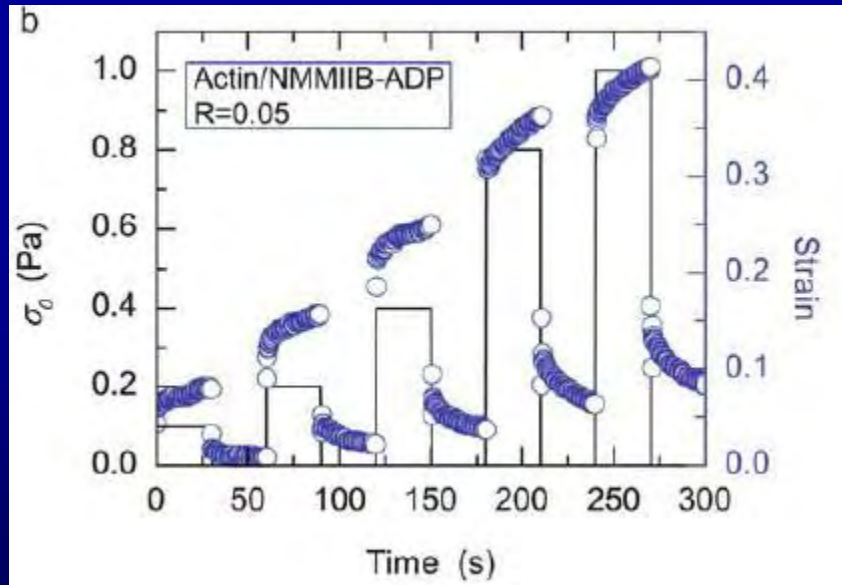
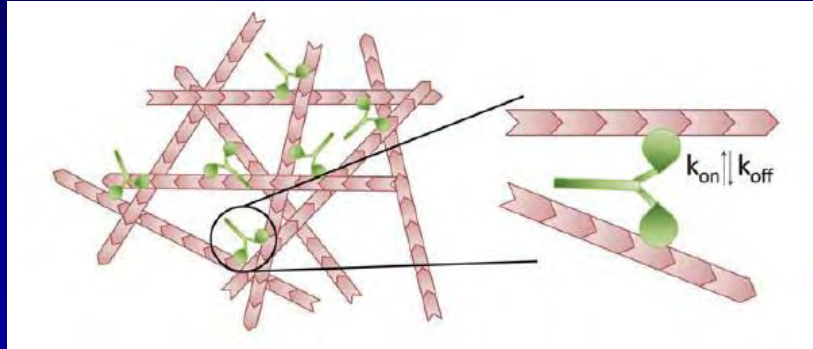


Solid-like behavior

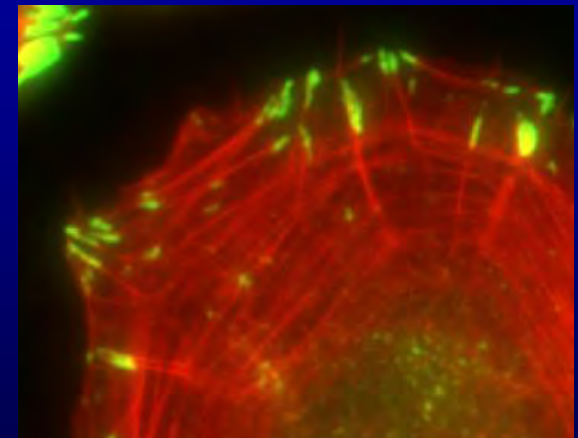
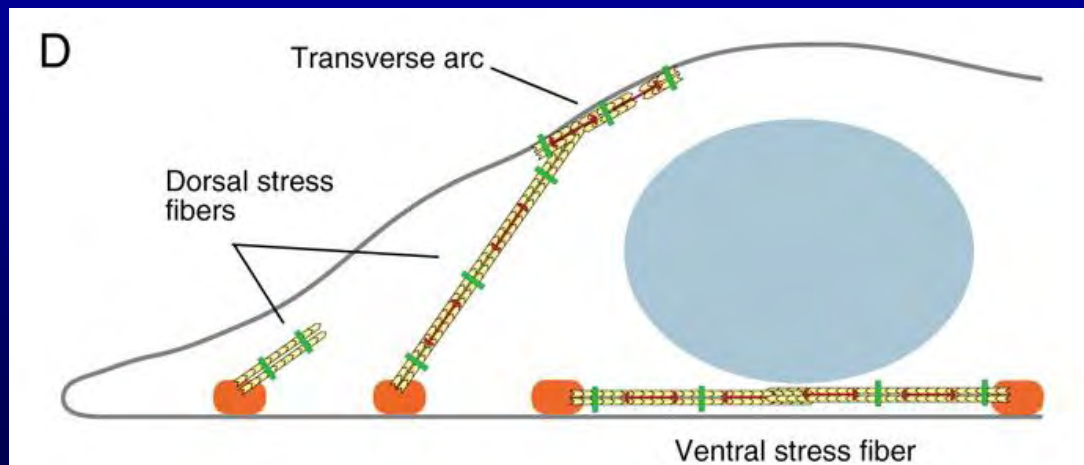
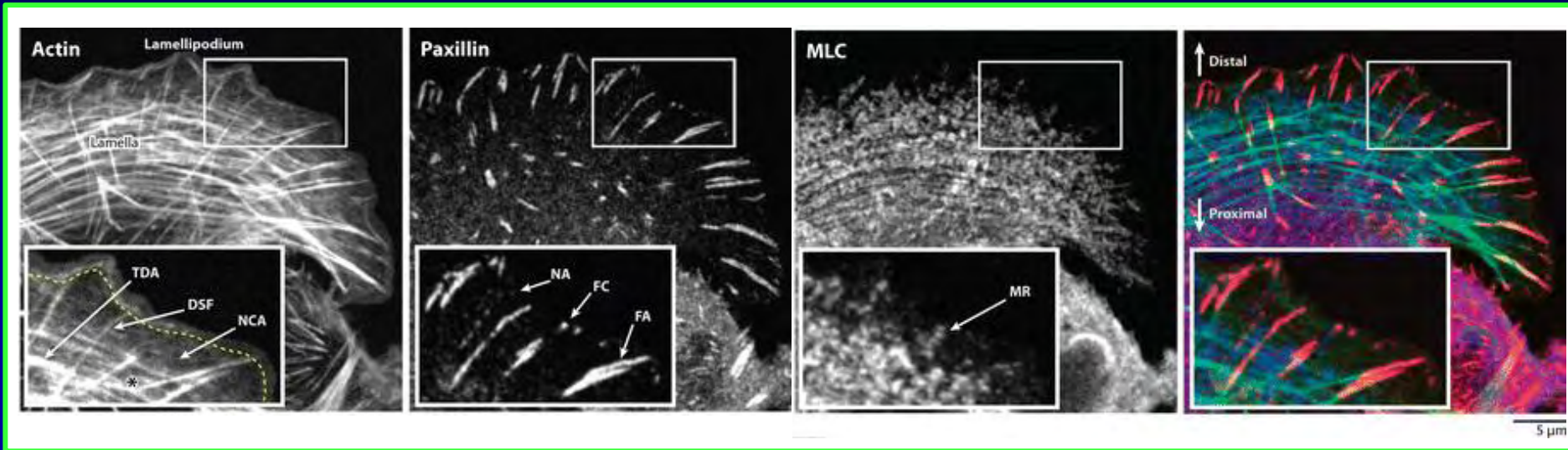
Contractile Lamellar Networks Insensitive to Substrate Stiffness



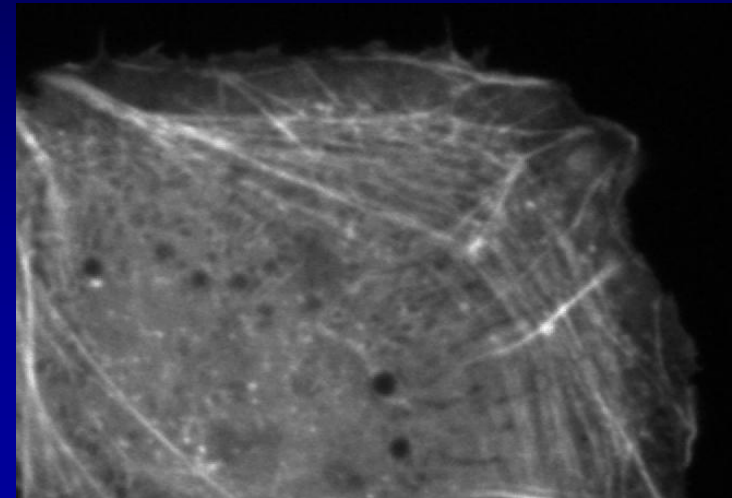
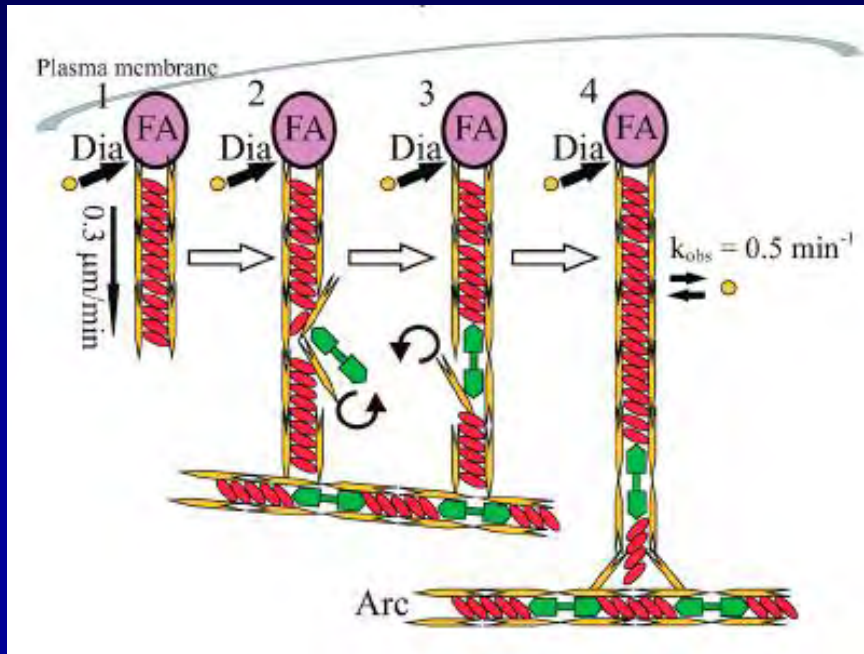
Prestress increases the Maxwell Relaxation time of Actomyosin Networks



Dorsal Stress Fibers thought to link adhesions to Lamellar Actin

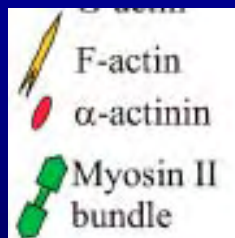


Stress fiber elongation occurs via mDia1 driven actin polymerization and myosin-dependent retrograde flow



Elongation rate = 5 nm/s

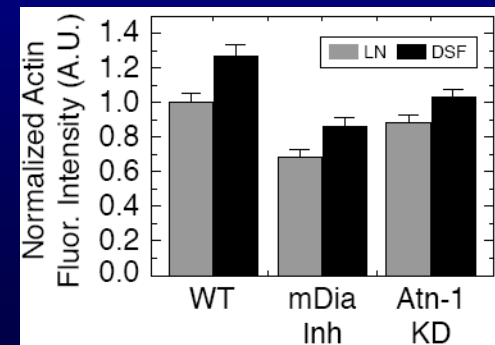
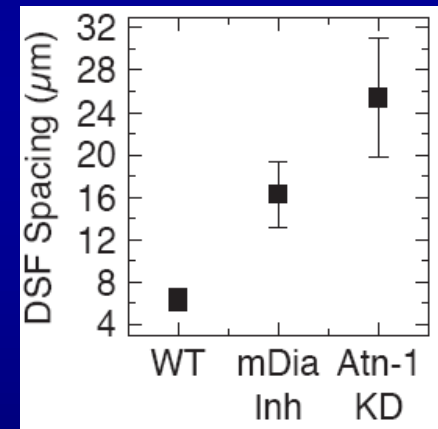
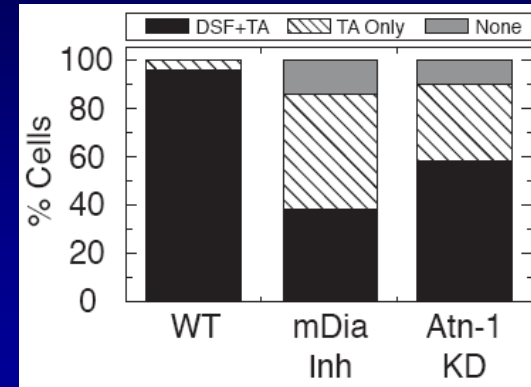
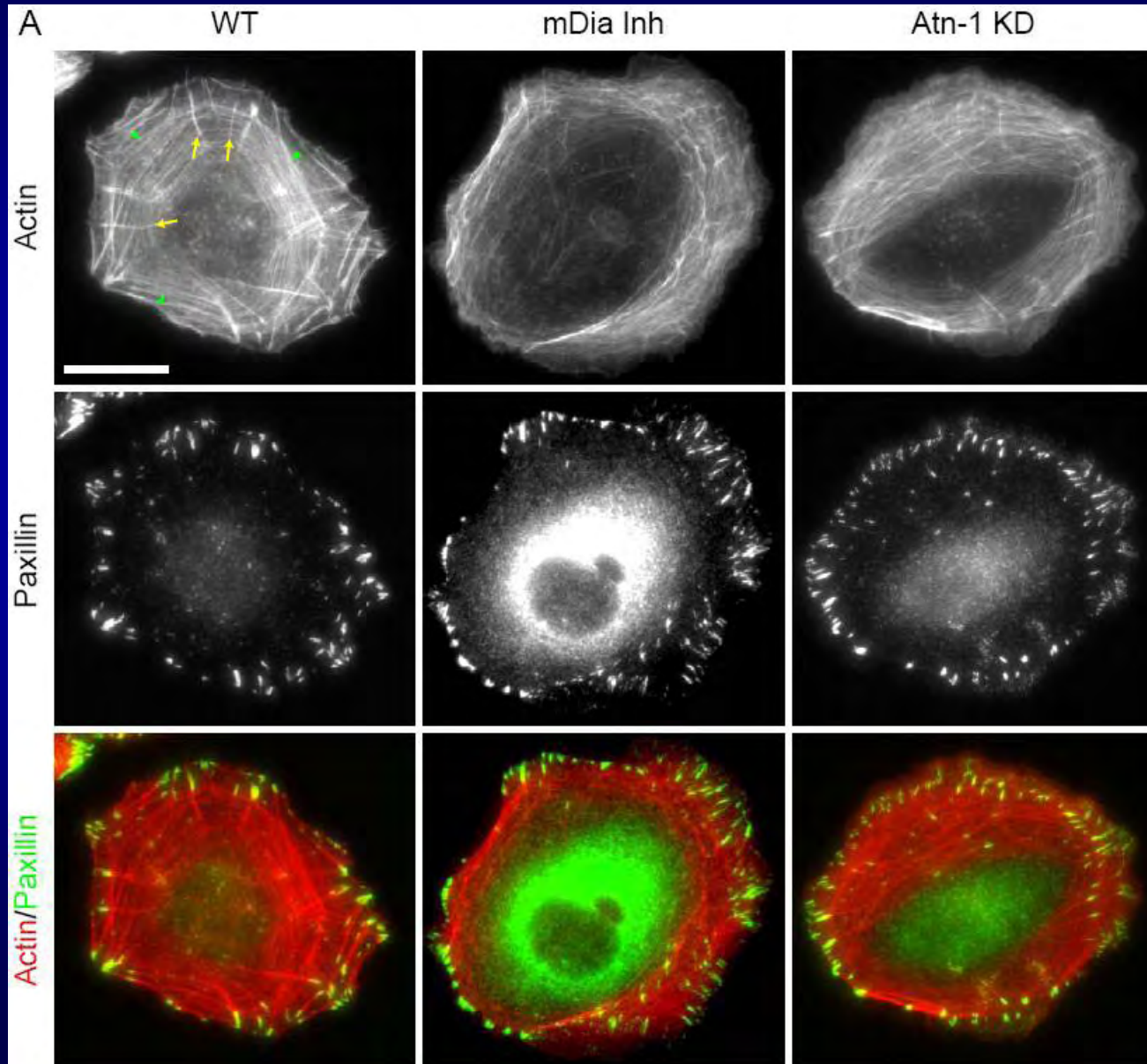
Hotulainen and Lappalainen, JCB 2006



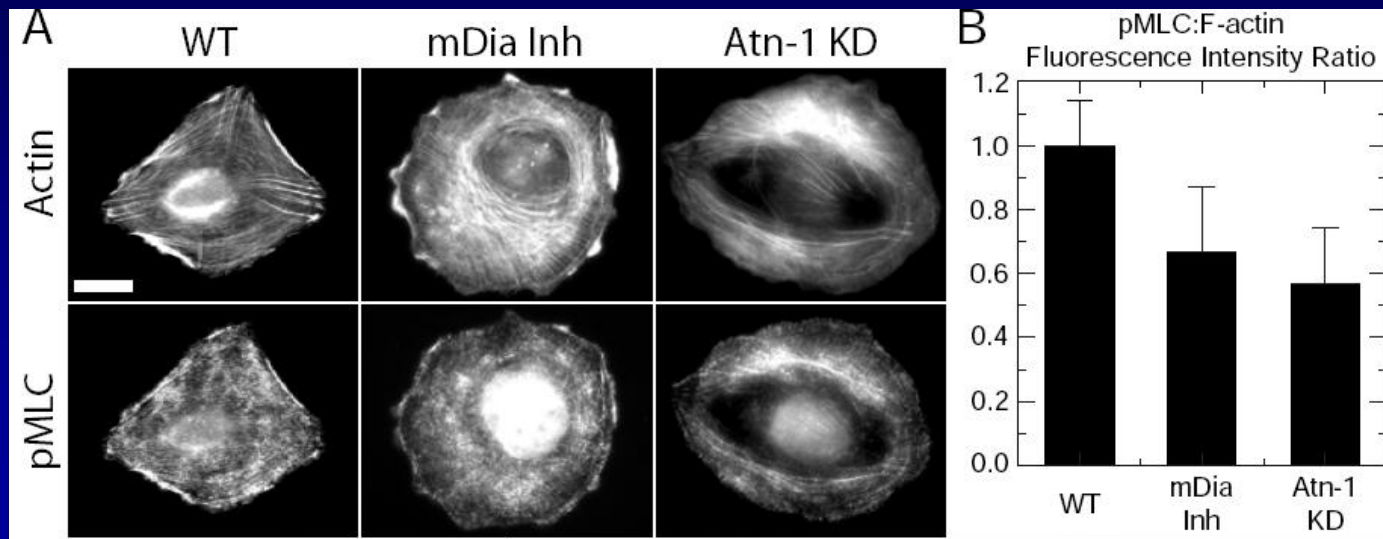
Myosin II – generates force and retrograde movement, cross-links F-actin
mDia 1 – actin filament nucleator
α-actinin – actin crosslinking protein

What are the consequences of inhibiting stress fiber assembly?

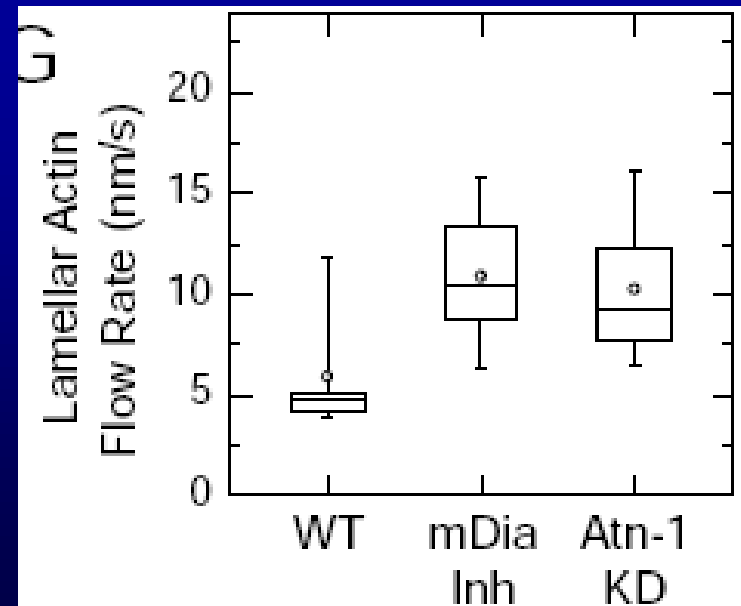
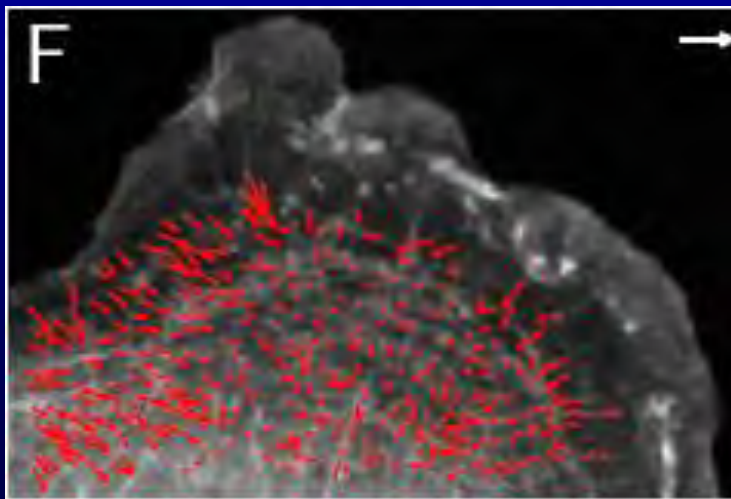
Formin and α -actinin required for stress fiber assembly



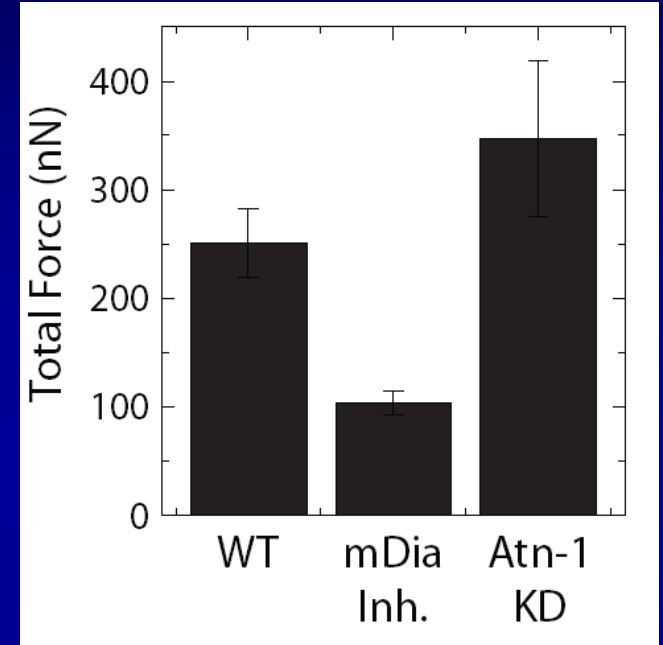
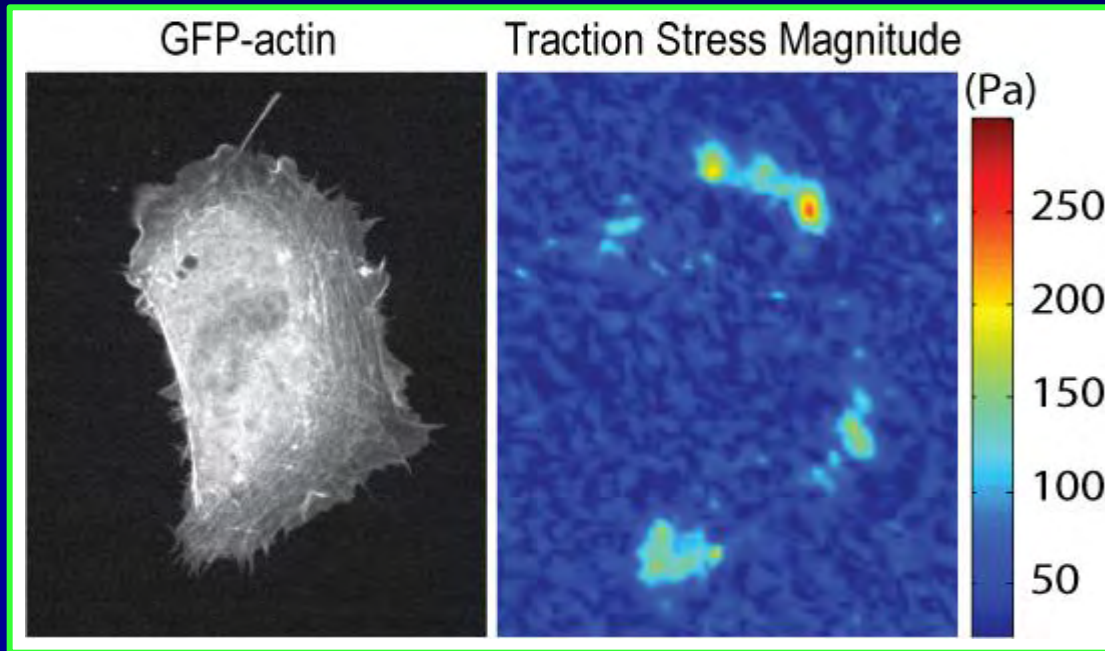
Eliminating stress fibers results in rapid lamellar actin flow



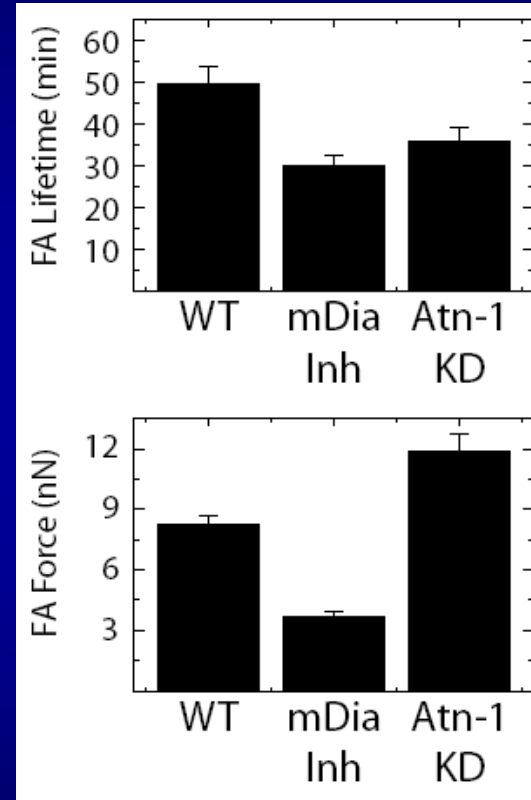
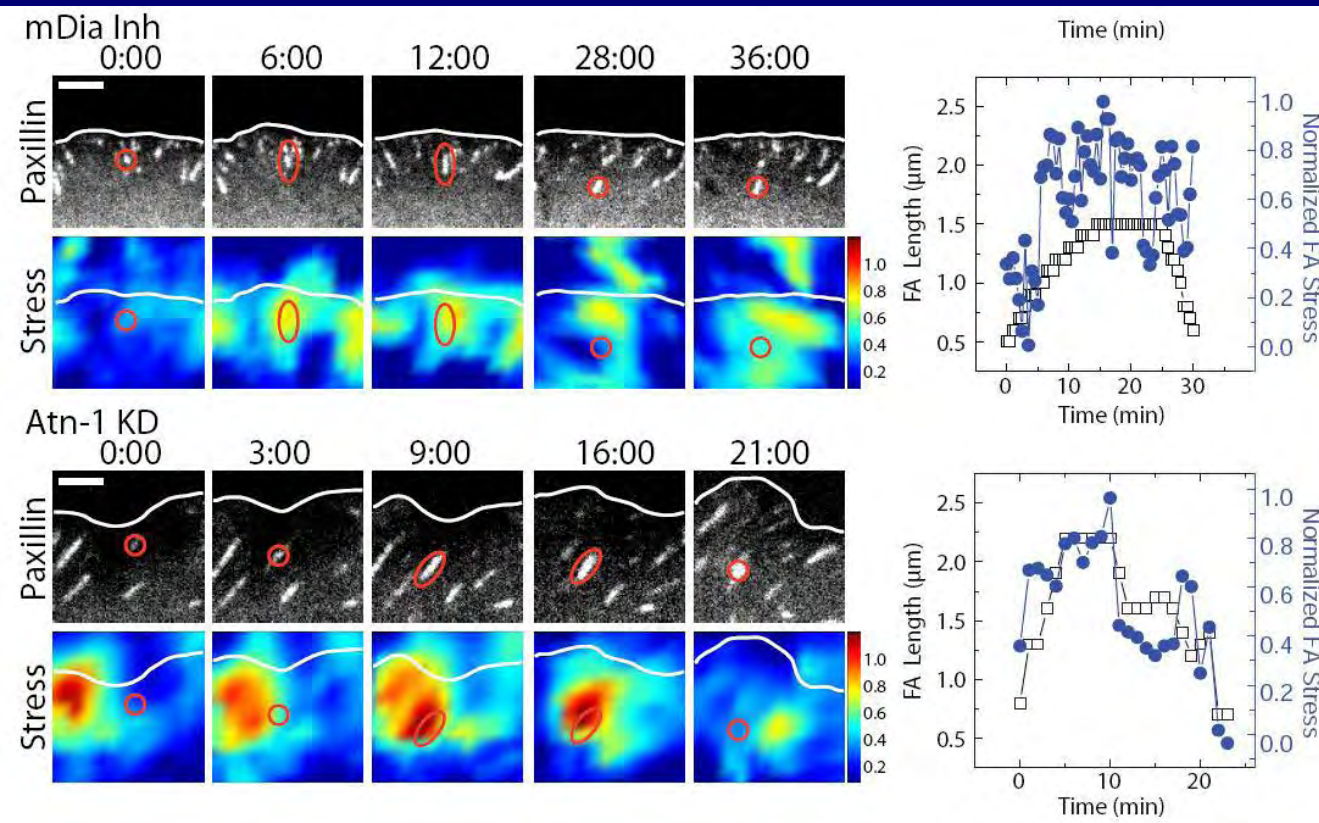
Actin Flow Vectors



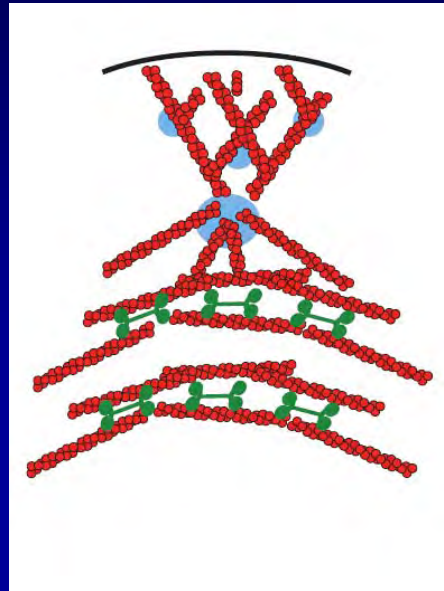
Large Traction Forces Generated in the Absence of Stress Fibers



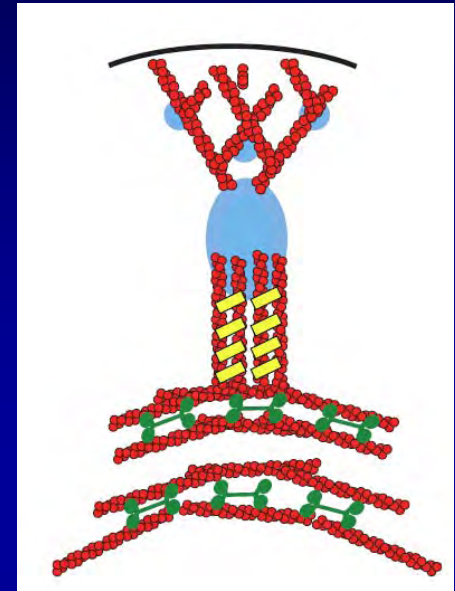
Tension build up at adhesions occurs in the absence of stress fiber assembly



Lamellar Network



Dorsal Stress Fibers



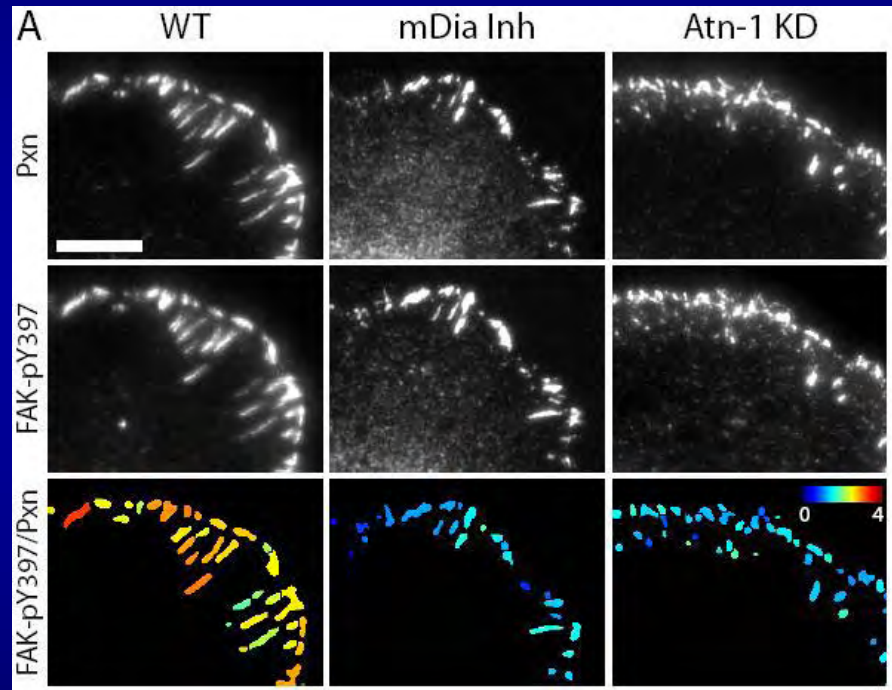
Traction Forces
Retrograde Flow
FA lifetime
FA Morphology
FA Composition
ECM Remodeling

1-12 nN
10 nm/s
30 min
small

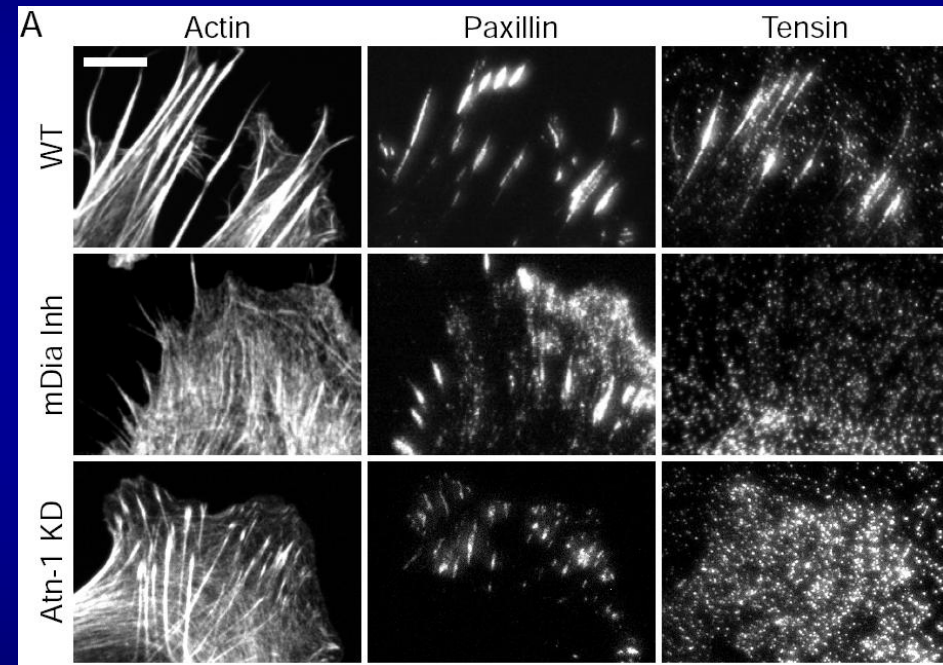
1-12 nN
5 nm/s
50 min
large

Inhibiting Stress fiber assembly impairs FA compositional maturation

Reduced pY397 FAK/pY 31 Pxn

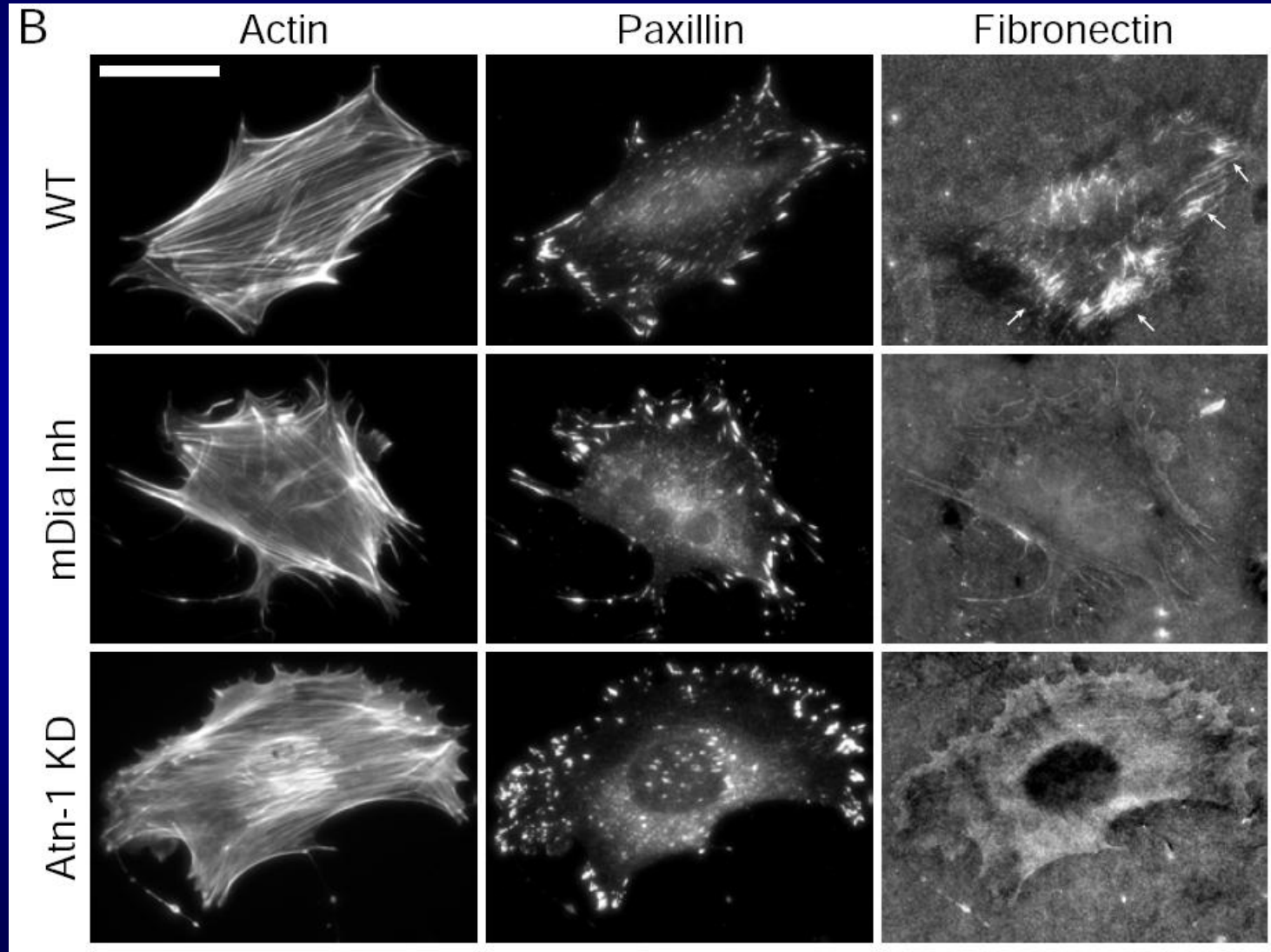


Impaired formation of fibrillar adhesions

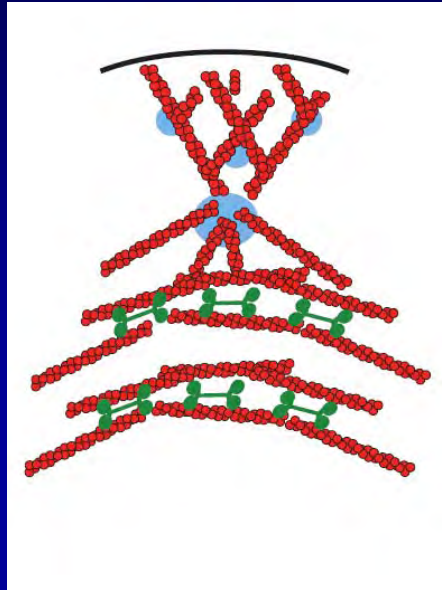


Tension is insufficient to mediate compositional maturation of adhesions

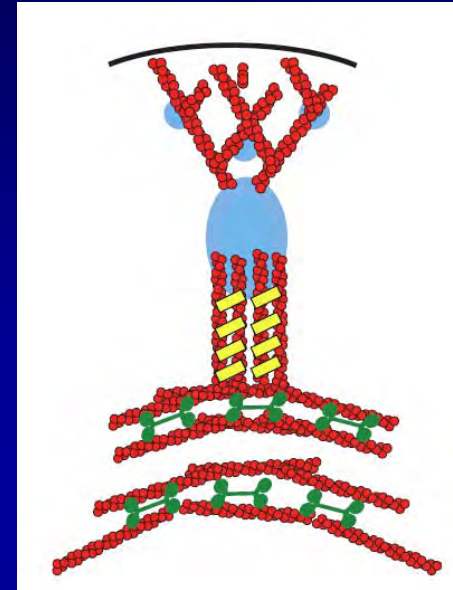
Inhibiting Stress Fiber Assembly Abolishes ECM remodeling



Lamellar Network



Dorsal Stress Fibers



Traction Forces
Retrograde Flow
FA lifetime
FA maturation
FN remodeling

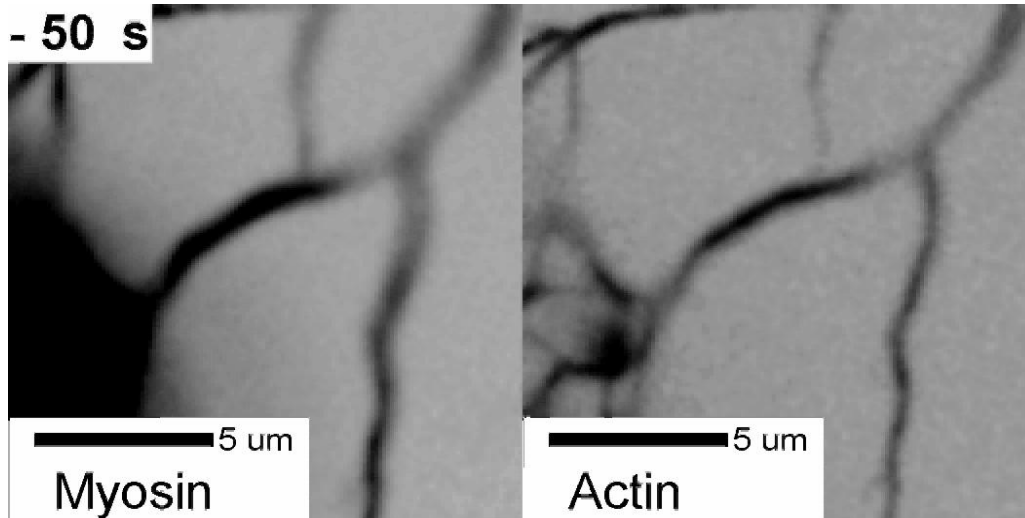
1-12 nN
10 nm/s
30 min
NO
NO

1-12 nN
5 nm/s
50 min
YES
YES

Summary

- Disordered Actomyosin networks generate large forces over rapid time scales
 - Rate of Force build up is insensitive to substrate stiffness
- Force-velocity relationship of contractile Lamellar Networks mimics myosin-II mechanochemistry
 - In Lamellar Networks, Contractile Elements add in parallel!

Reconstituted actomyosin *bundles* have contractile elements in series

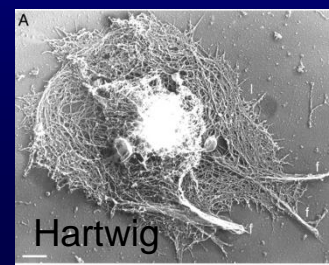


Thoresen et. al., BJ, 2011

linking adhesion plaques to
efficient force transmission
actin remodeling

ers do not reflect the extent of

e



Cell Biology

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Jonathan Stricker (Physics)
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Funding: Burroughs Wellcome Career Award, NIH Director's Pioneer Award, Packard Foundation