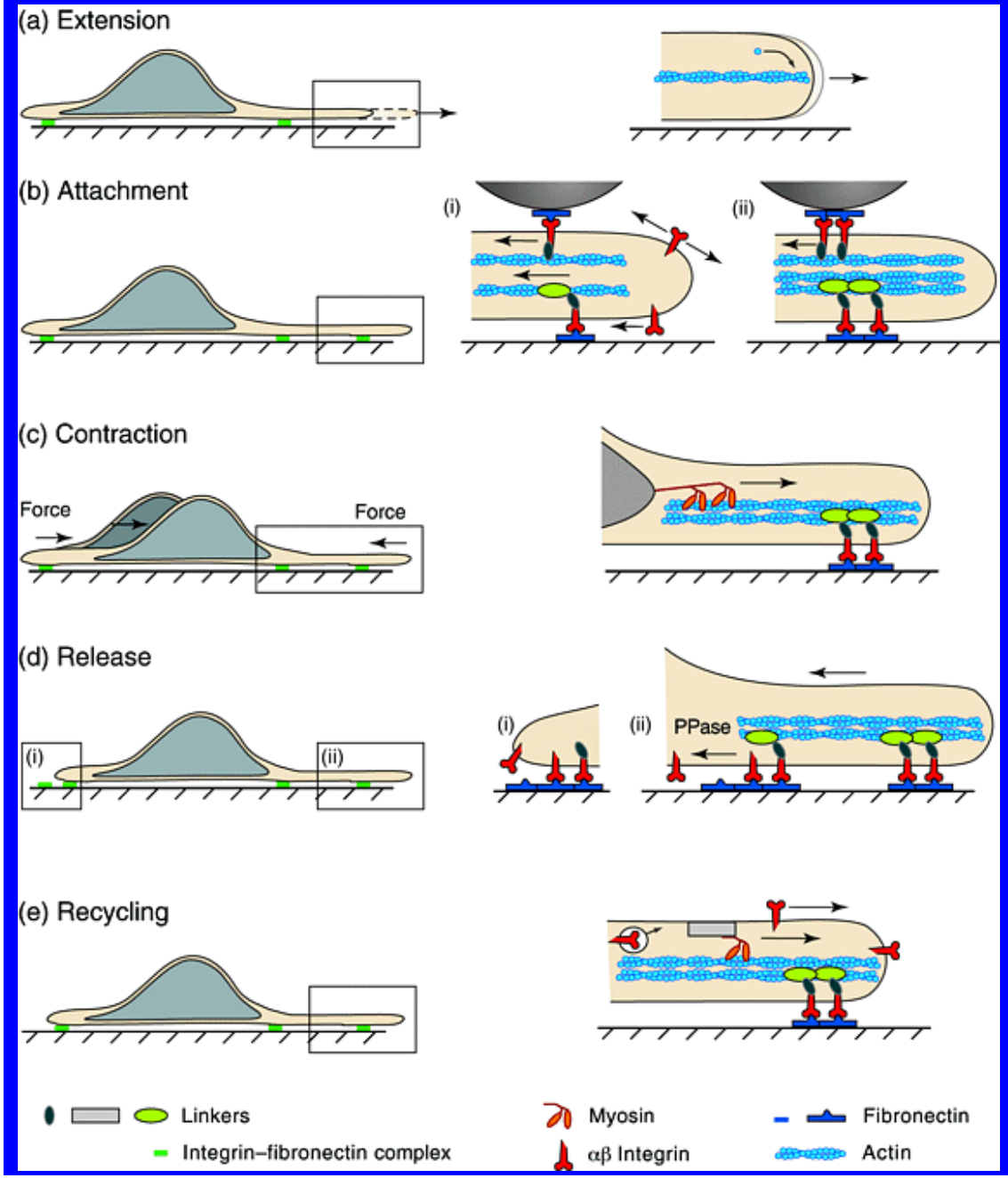
Mechanical Sensing in Normal and Transformed Cells

- What differentiates a formed organism from a sphere of cells is the ability to apply and sense forces at specific places and times
- Rapid neuronal sensing of force is through ion channels. Turgor in tissues and long-term forces are sensed by cytoskeletal-based mechanisms.
- Transformation is described as the ability to grow on soft agar, i.e. in the absence of force.

Cell migration: a cyclic process of force generation

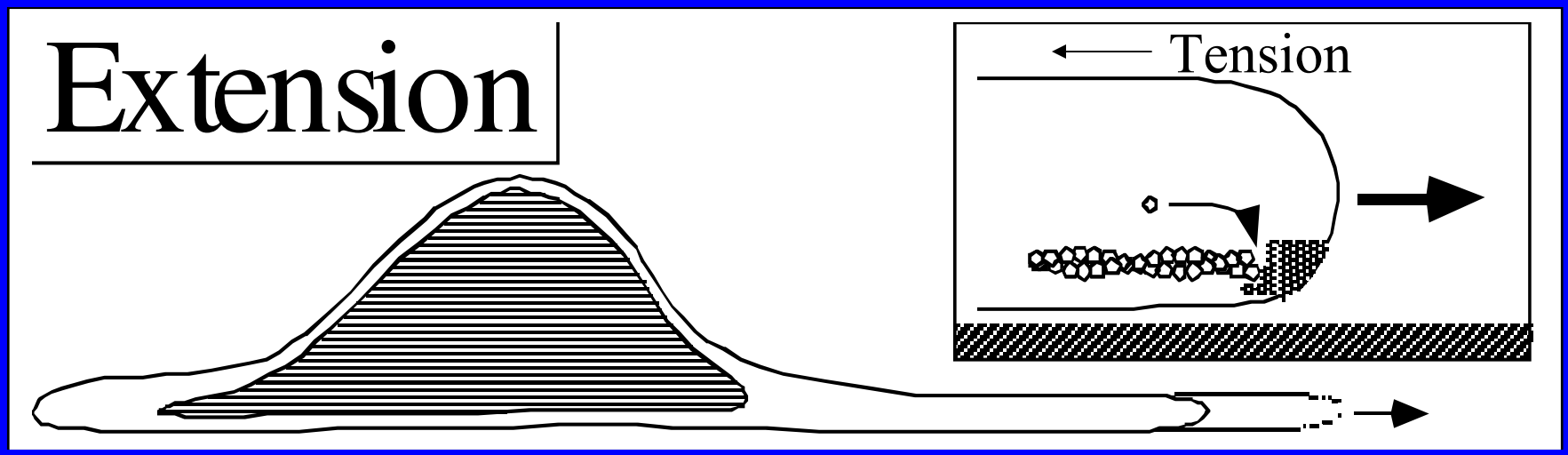


Membrane-Cyto. Adhesion Resists Extension α PIP2. Sheetz, Nature Rev. MCB. 2:392 (2001)

Extracellular Matrix Adhesion Linked to Force Generation by Rearward Flow

How do cells locally transduce Matrix rigidity into a signal used to direct cell edge extension?

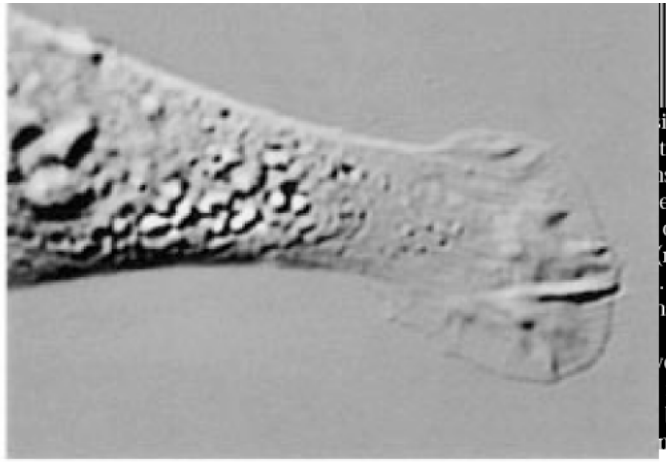
Apparent Membrane Tension Resists Extension by Actin and Tension α [PIP2]

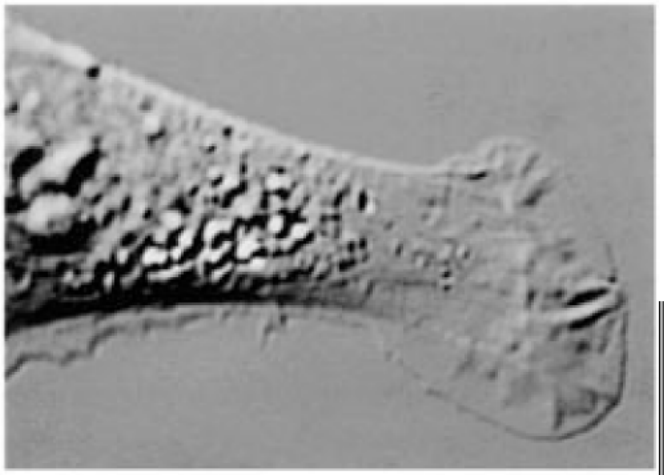


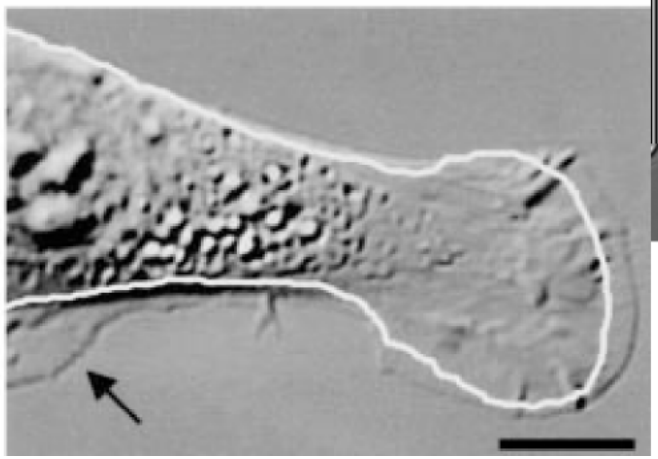
Reviewed: Sheetz, Nature Rev. MCB. 2:392 (2001)

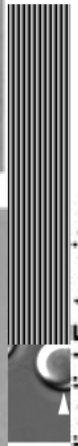
counted, normalit
number of lame

Reagents that Increase Membrane Area Increase Lamellipodial Extension

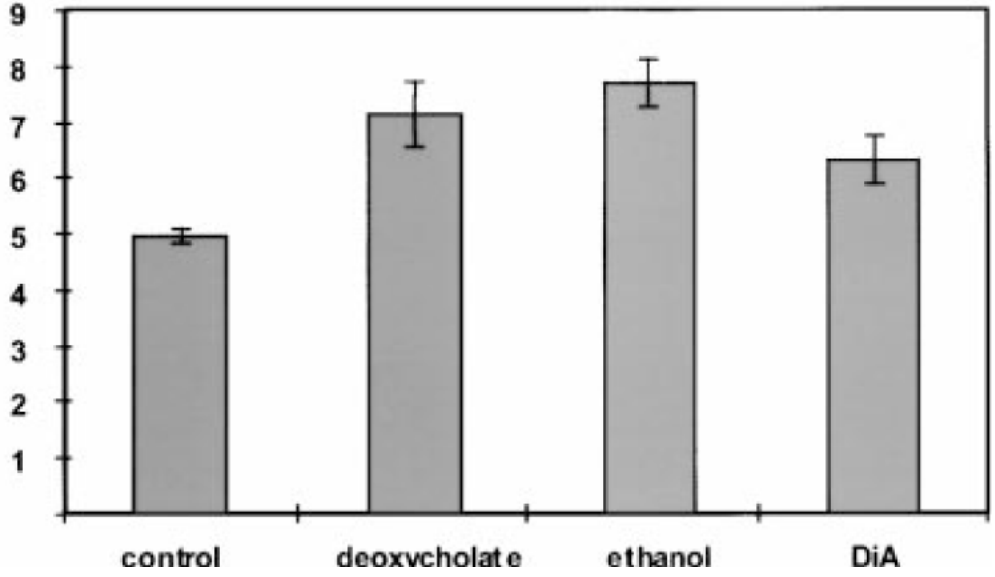


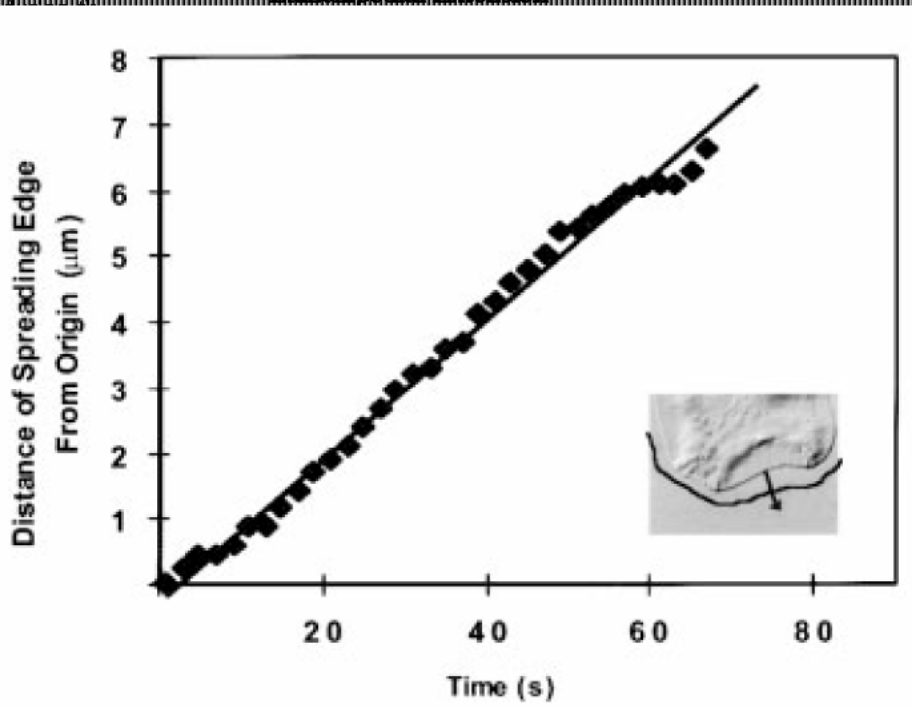


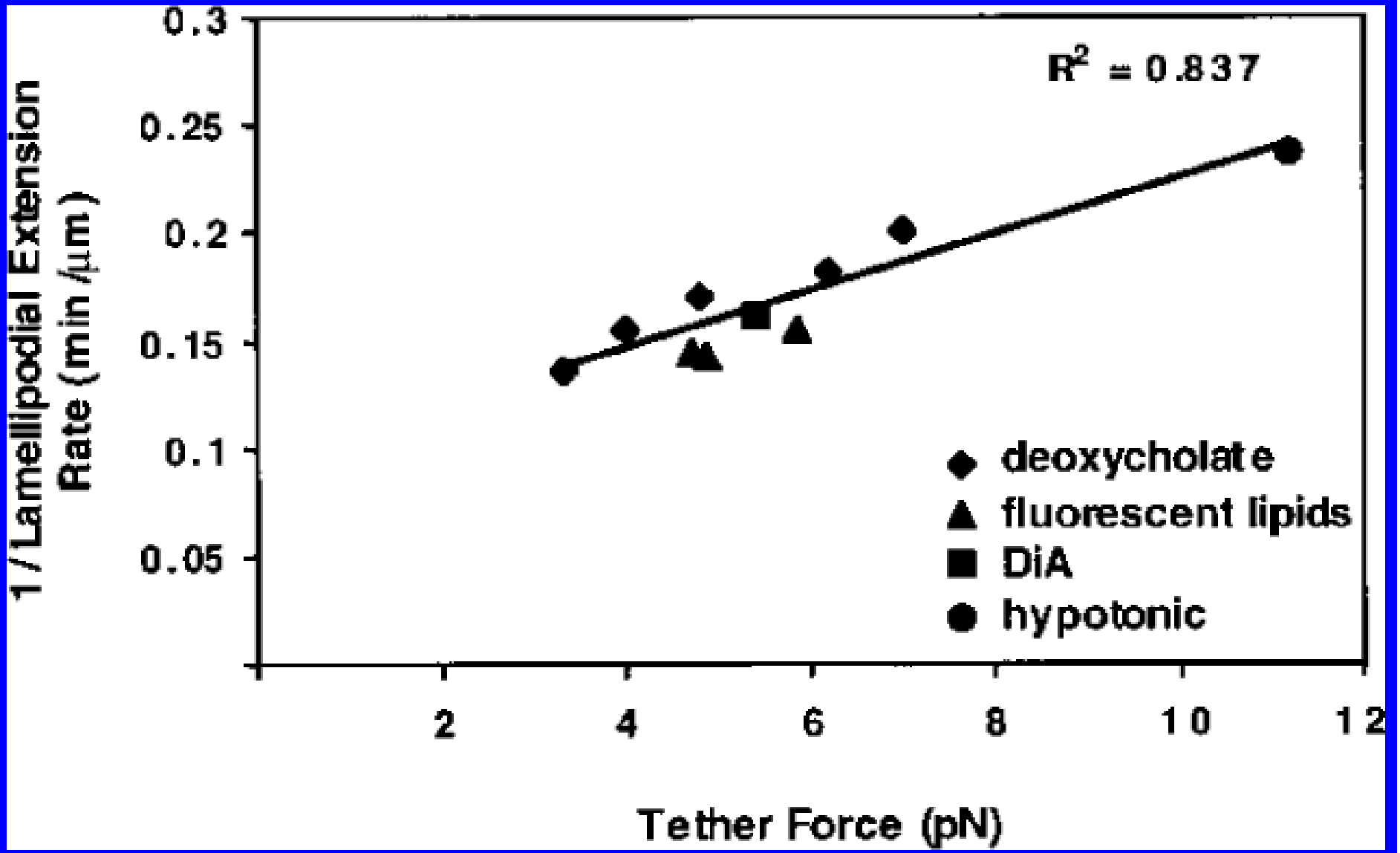




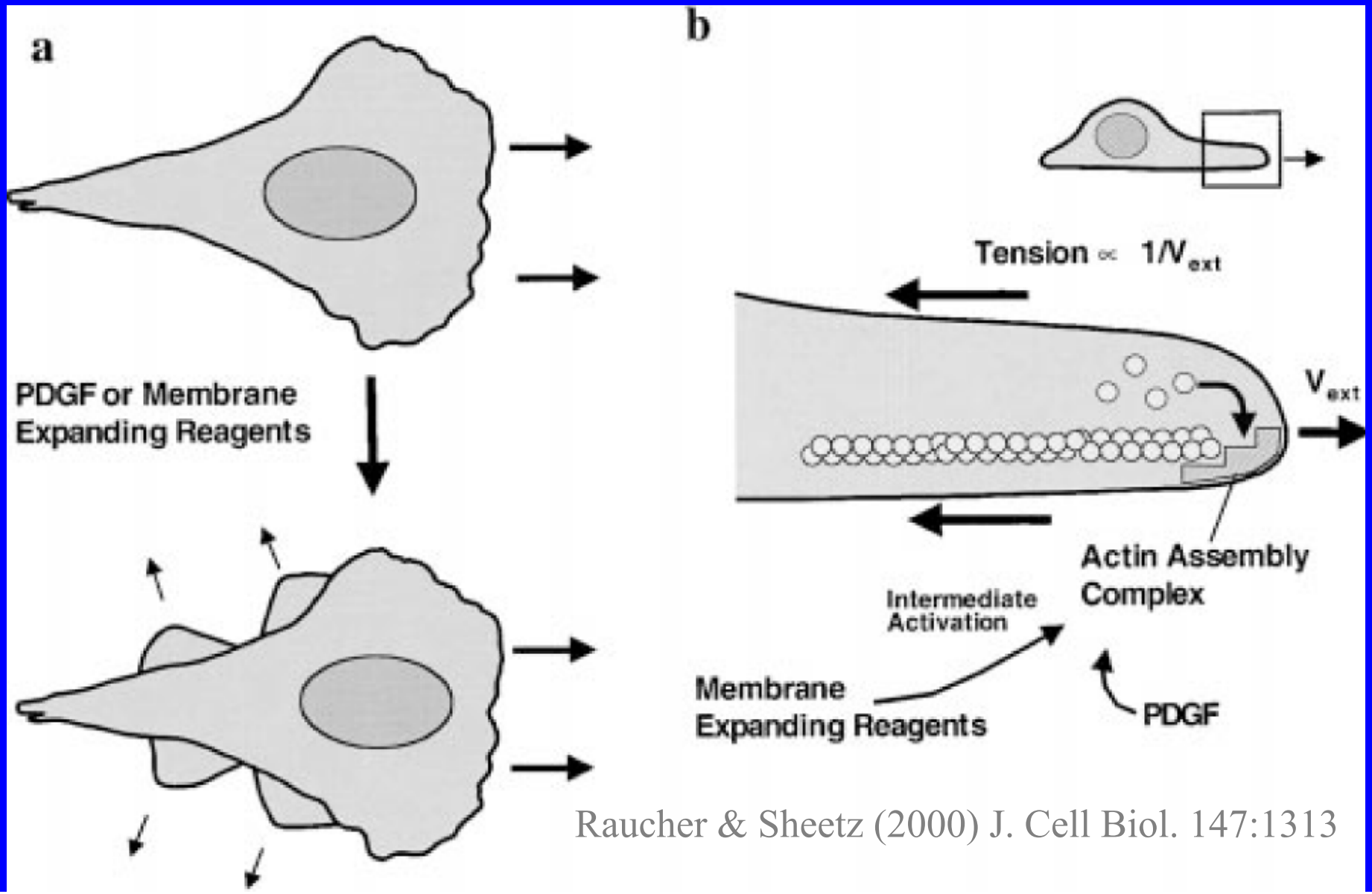
Lamellipodial Extension Rate(μm/min)






0.3
0.25
0.2
0.15
0.1
0.05
1 / Lamellipodial Extension Rate (min /μm)
R² = 0.837
deoxycholate
fluorescent lipids
DiA
hypotonic
2
4
6
8
10
12
Tether Force (pN)

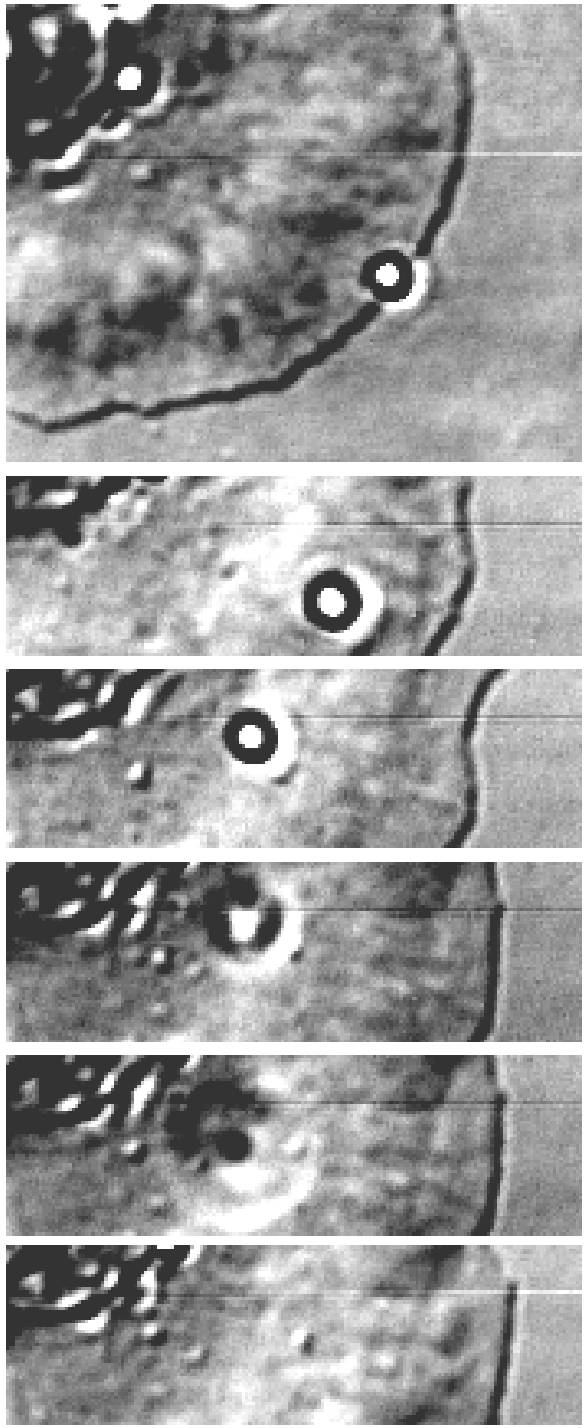
Membrane Load Alters Activation and Rate of Actin Assembly



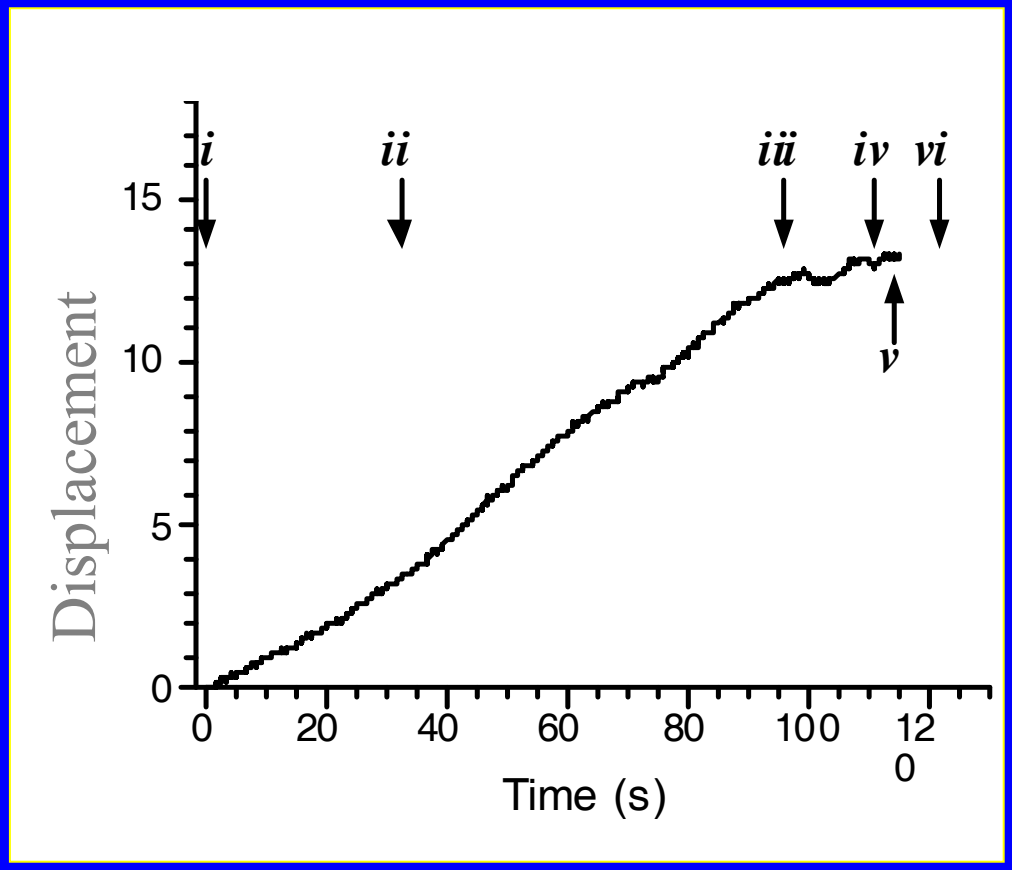
Raucher & Sheetz (2000) J. Cell Biol. 147:1313

Membrane Tension: Physical Control of Cell Functions

- Adhesion of membrane to the cytoskeleton develops tension that provides general regulation of endocytosis rate (surface-area/volume ratio), motility, and resealing
- Model of lipid-dependent membrane-cytoskeleton adhesion
- PIP2 is major lipid controlling membrane-cytoskeleton adhesion
- Tension is too low to activate stretch channels



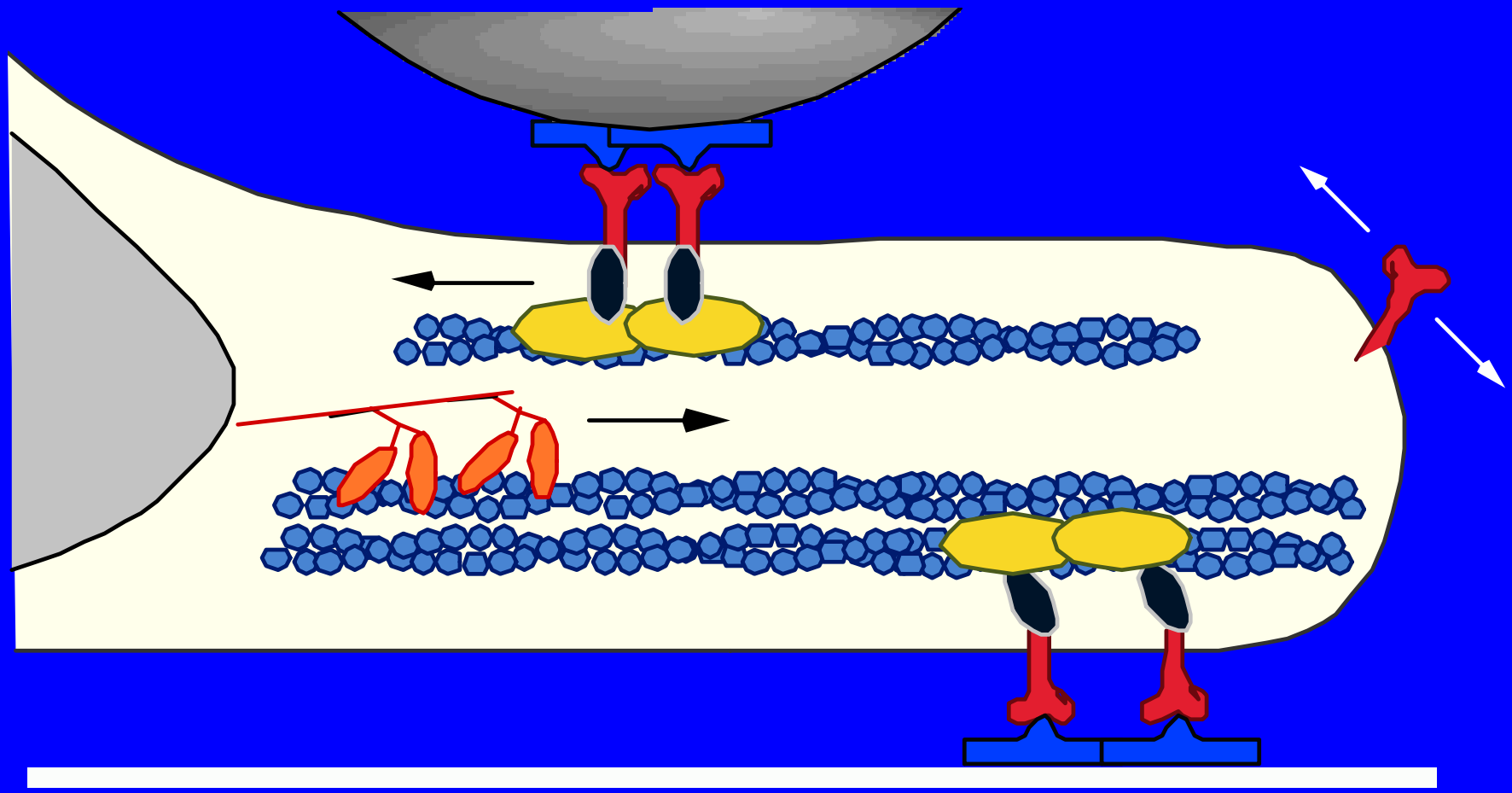
FN Bead Binding Movement and Release



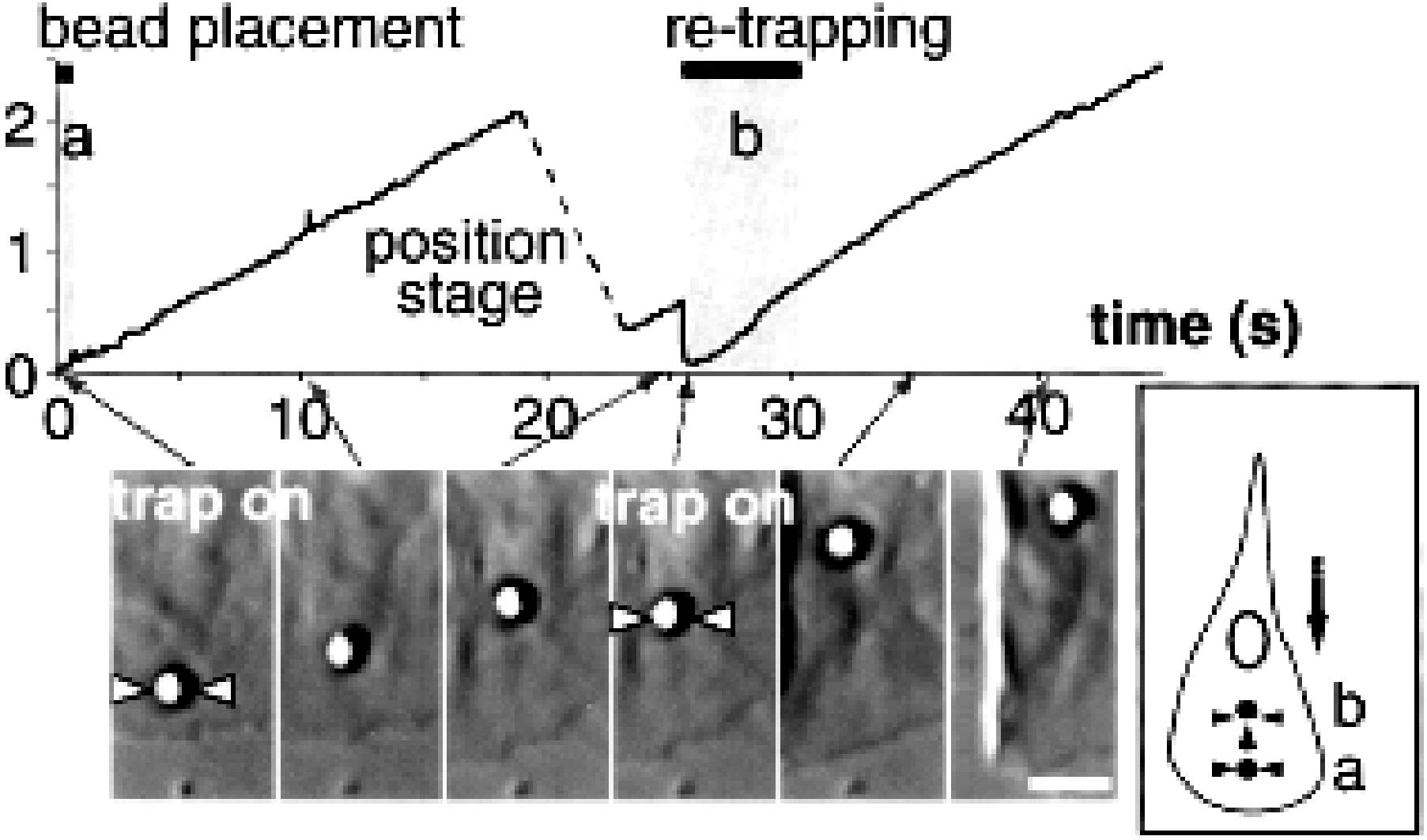
Nishizaka et al., PNAS 97:692 (1999)

How does a cell treat the ECM?

Attachment and Contraction

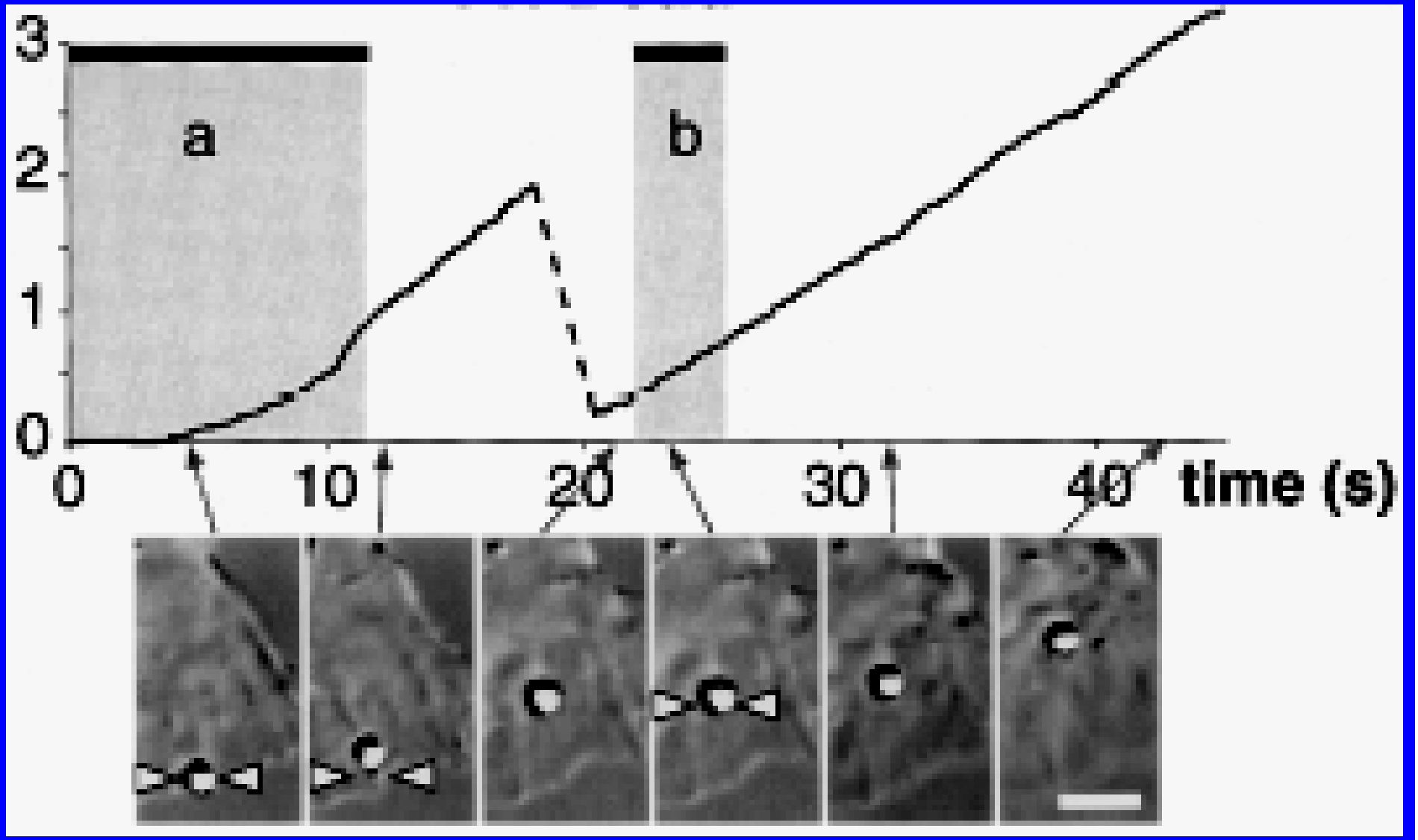


Unstressed Attachments

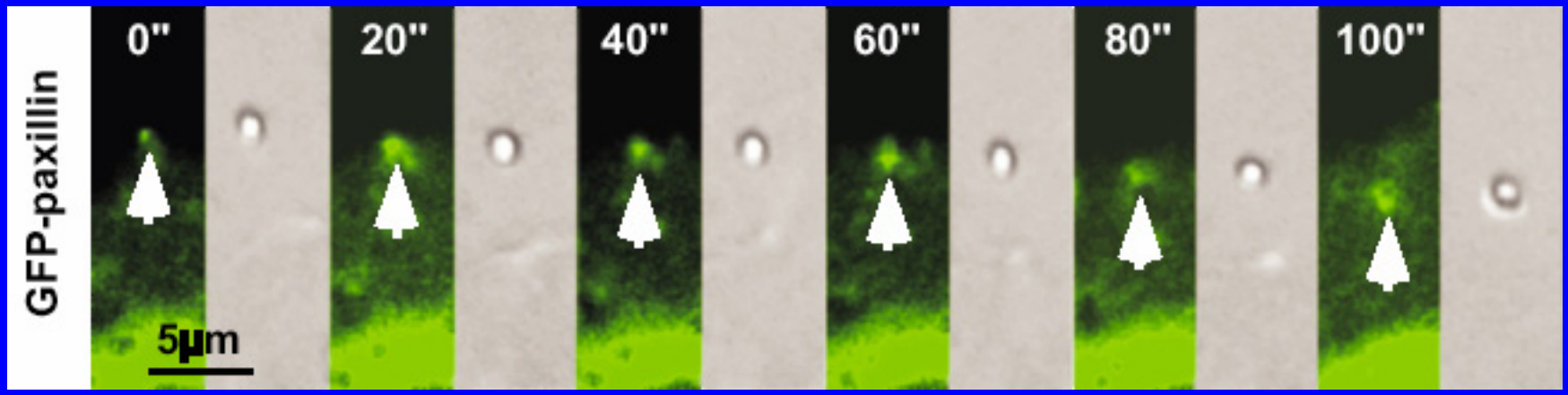


Choquet et al., Cell. 88:39 (1997)

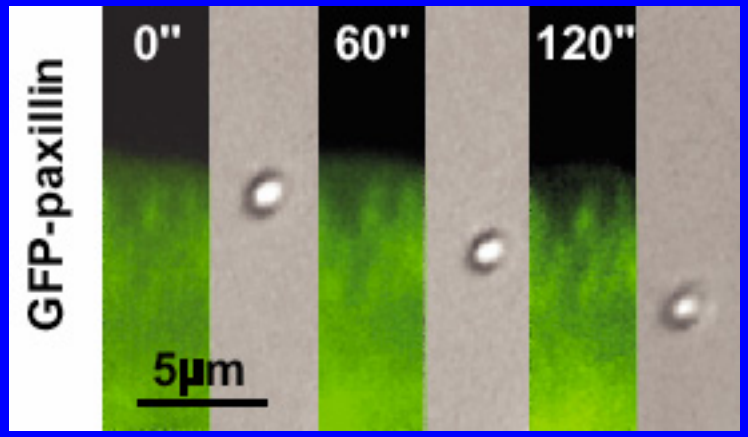
Force Causes Reinforcement



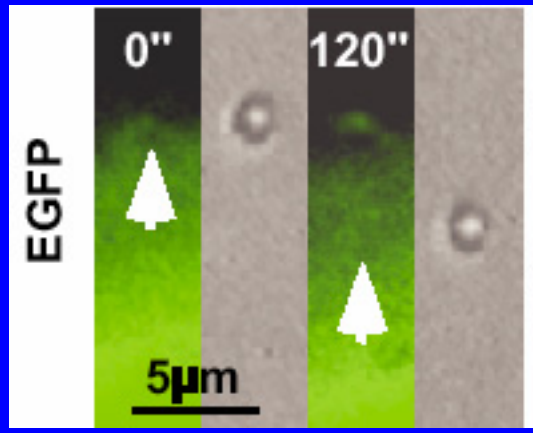
Force Causes GFP-Paxillin Accumulation in RPTPα+/+ with FN



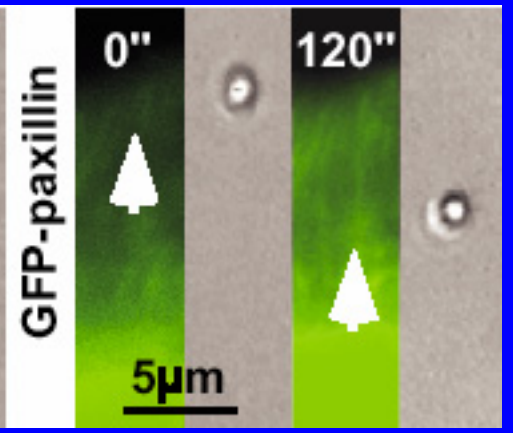
Without Force



FN Bead

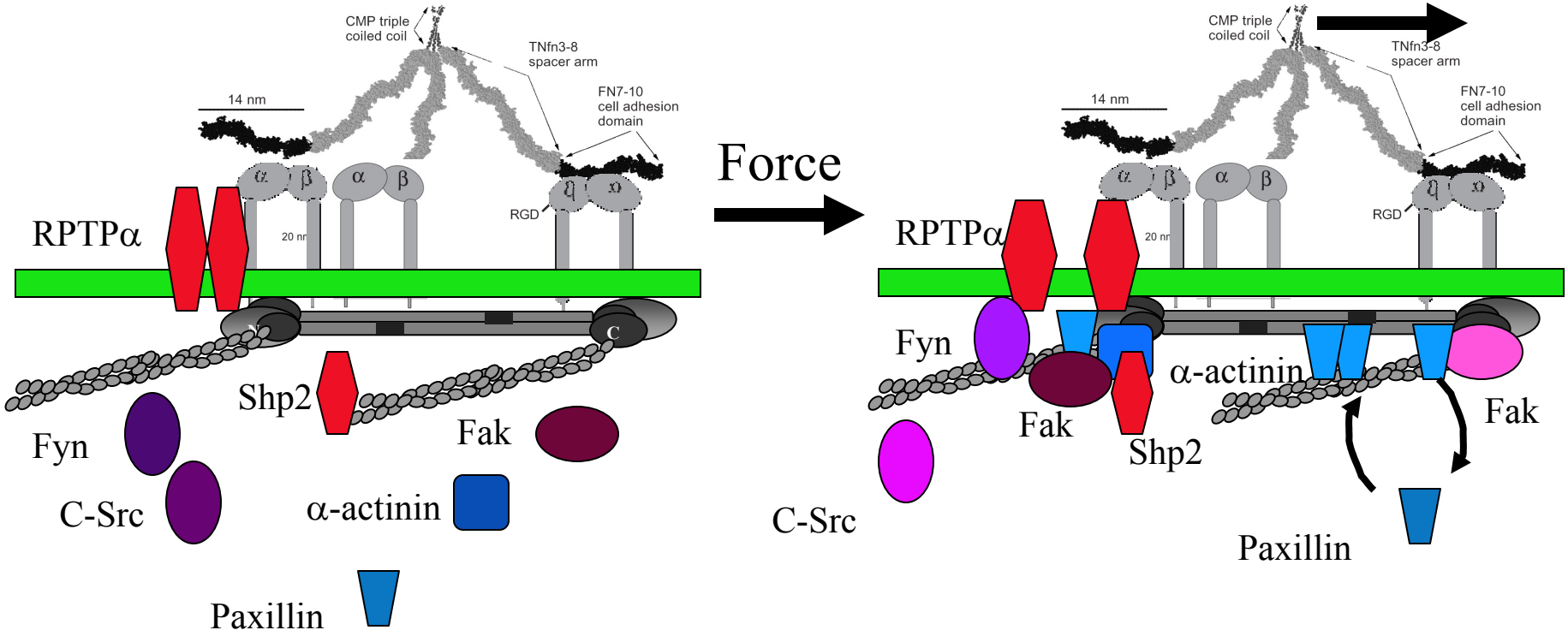


Con A Bead



Von Wichert et al., 2003. J. Cell Biol. 161:143-153

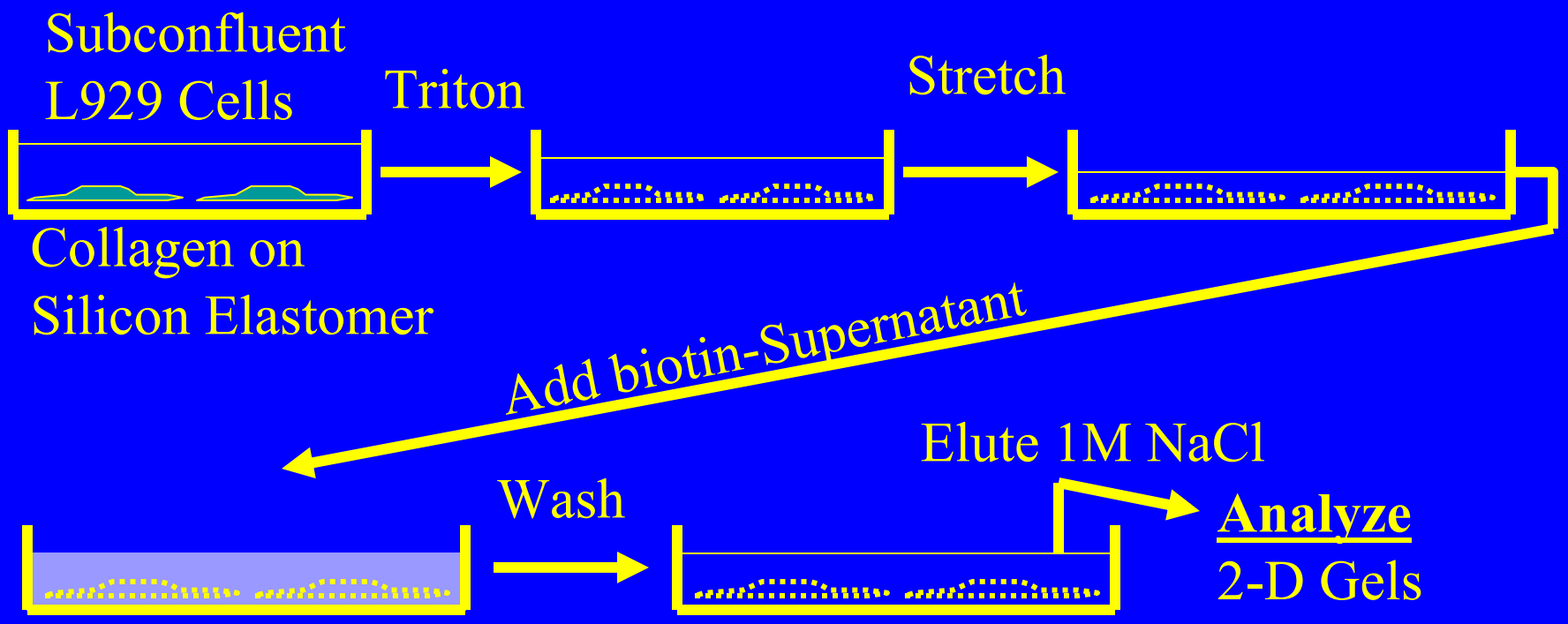
Force Effects in FN Contacts



Cytoskeleton Stretching Causes Focal Contact Protein Binding

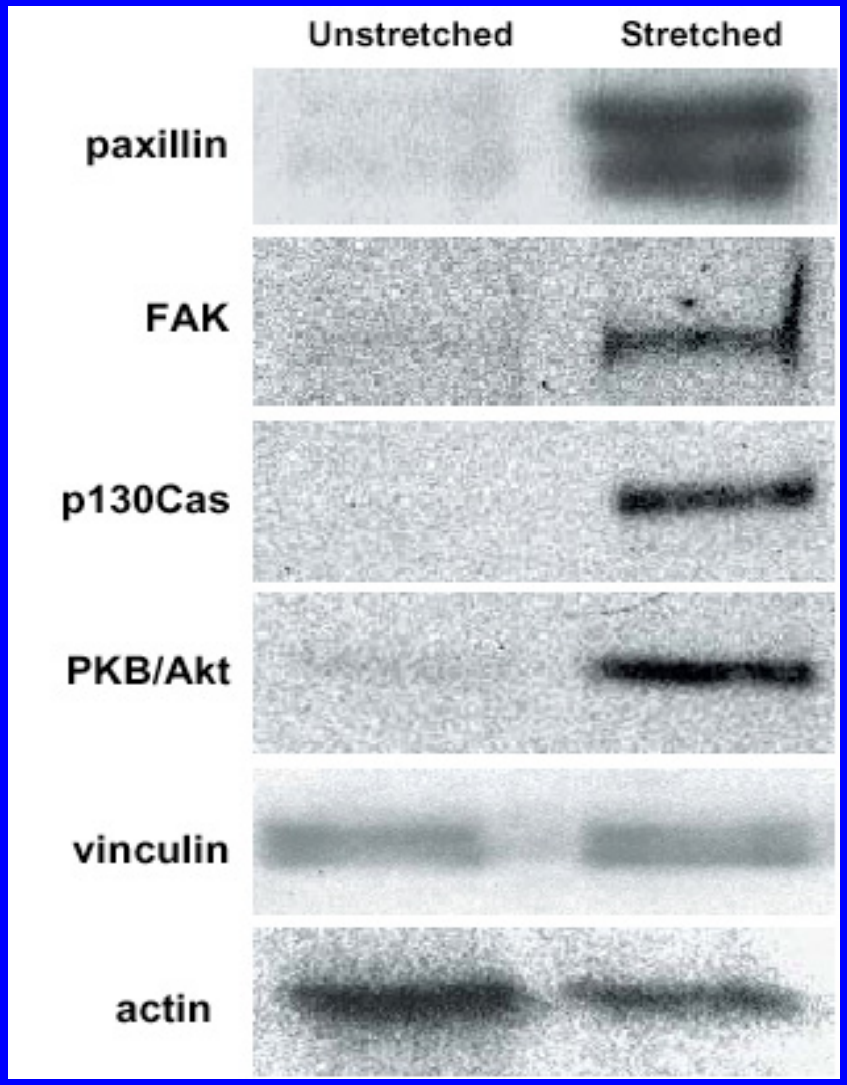
We tested the hypothesis that the cytoskeleton is being altered directly by force on matrix molecules using a stretchable silicon substrate and detergent-treated cells.

Stretching of Cytoskeletons

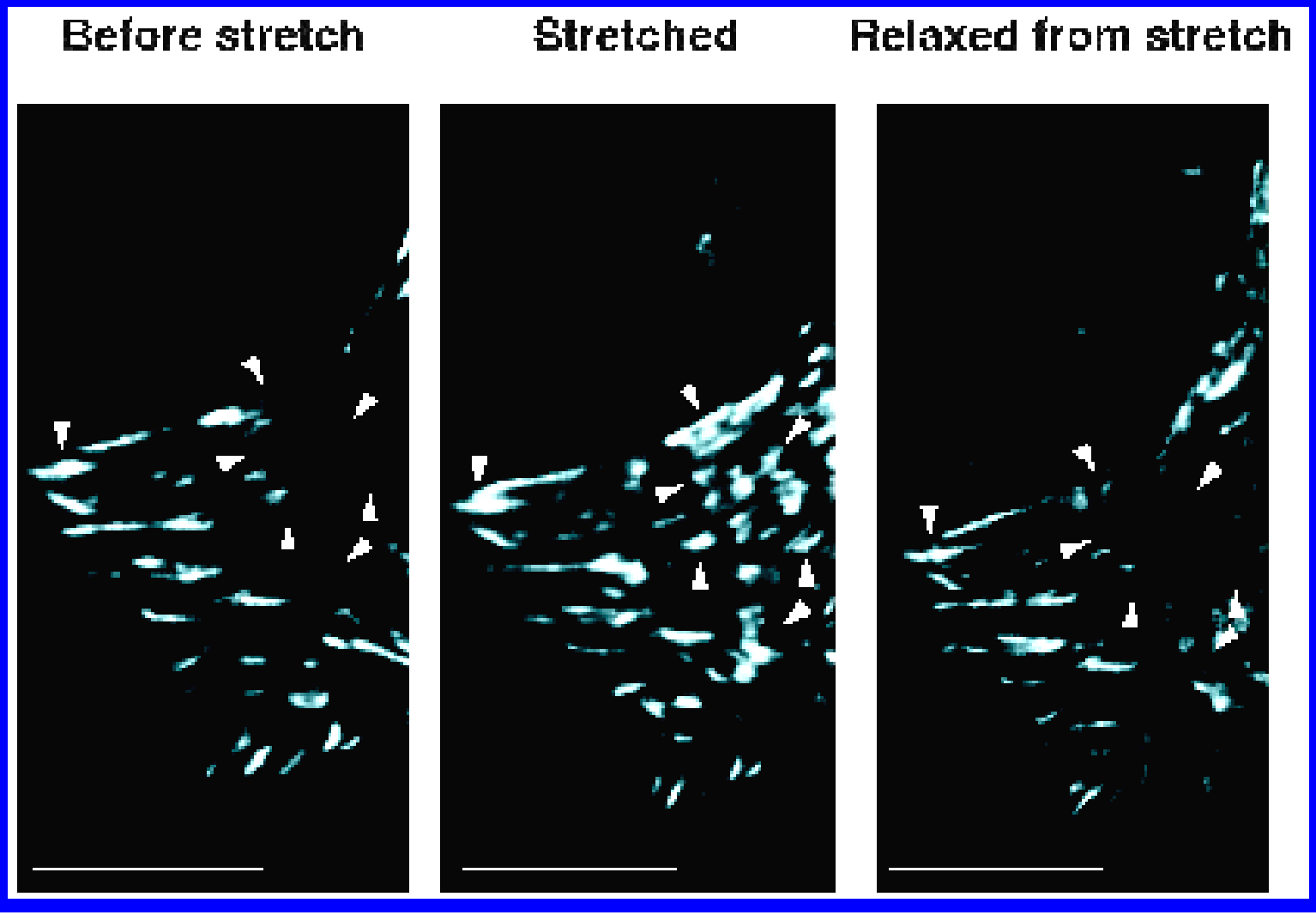


Sawada and Sheetz, JCB **156**:609 (2002)

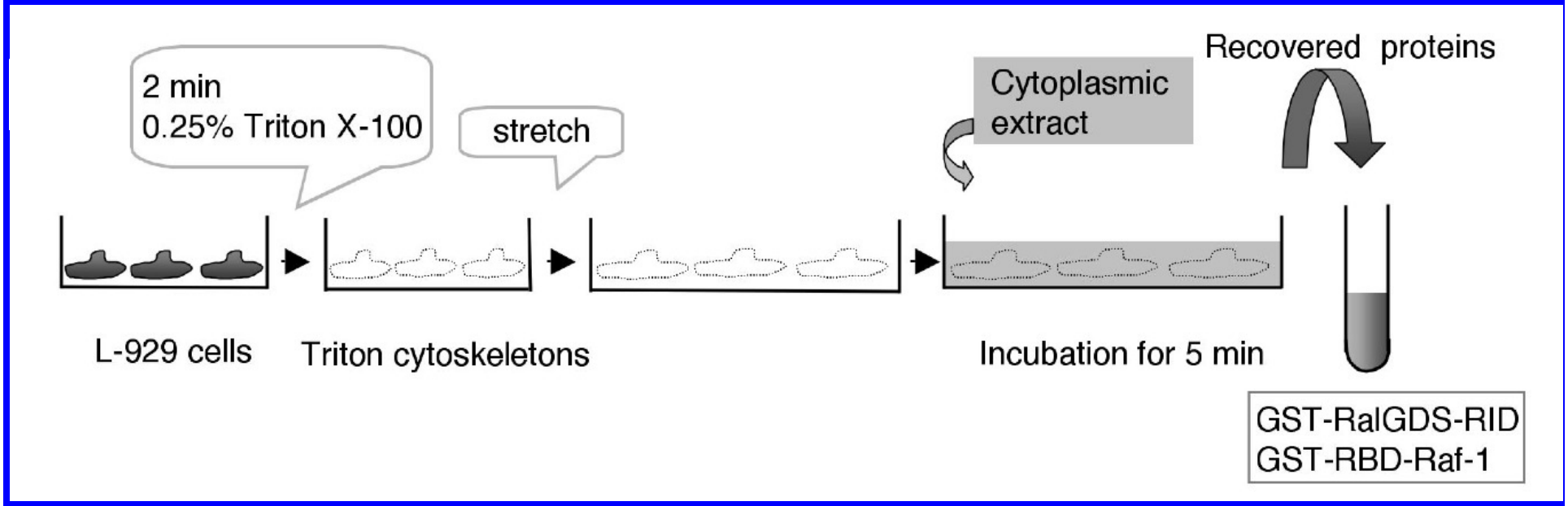
Antibody Analysis of Bound Proteins



GFP-Paxillin Stretch-Dep. Binding

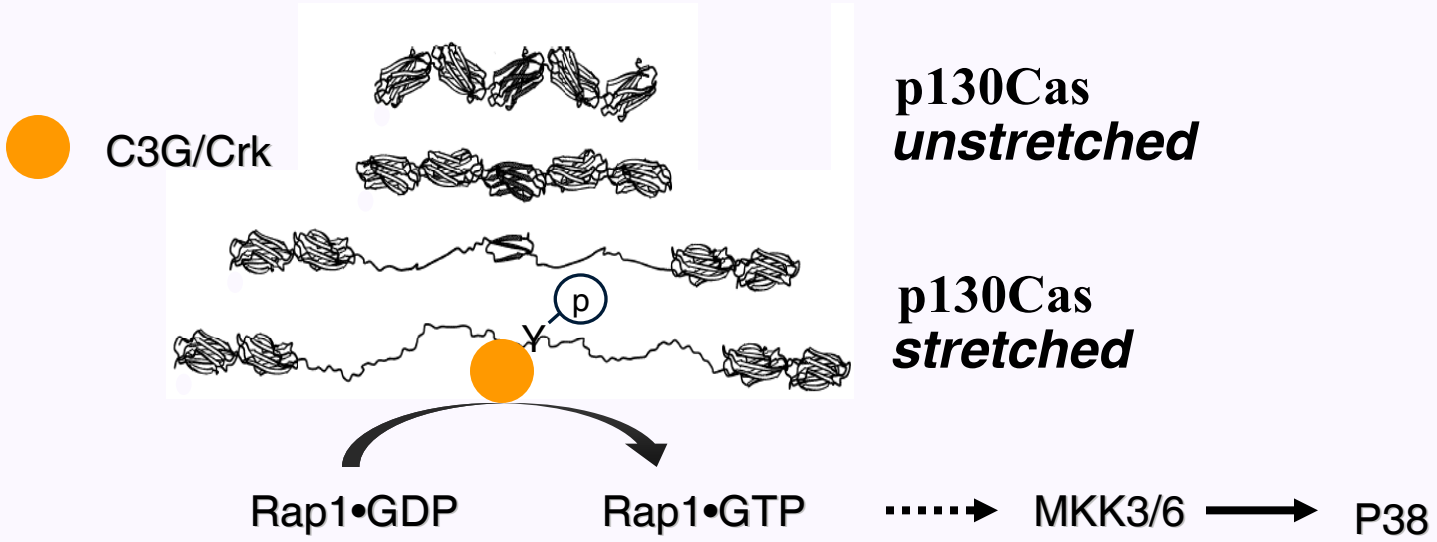


Protocol for Measuring G Protein Activation by Cytoskeletons

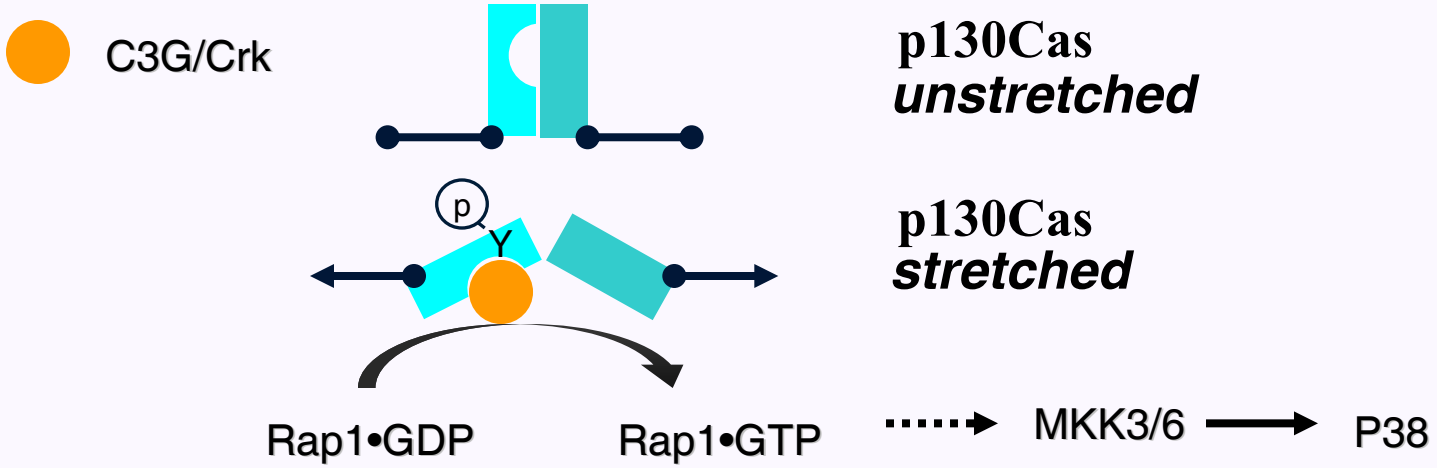


Tamada et al., 2004. Develop Cell, 7:709-18

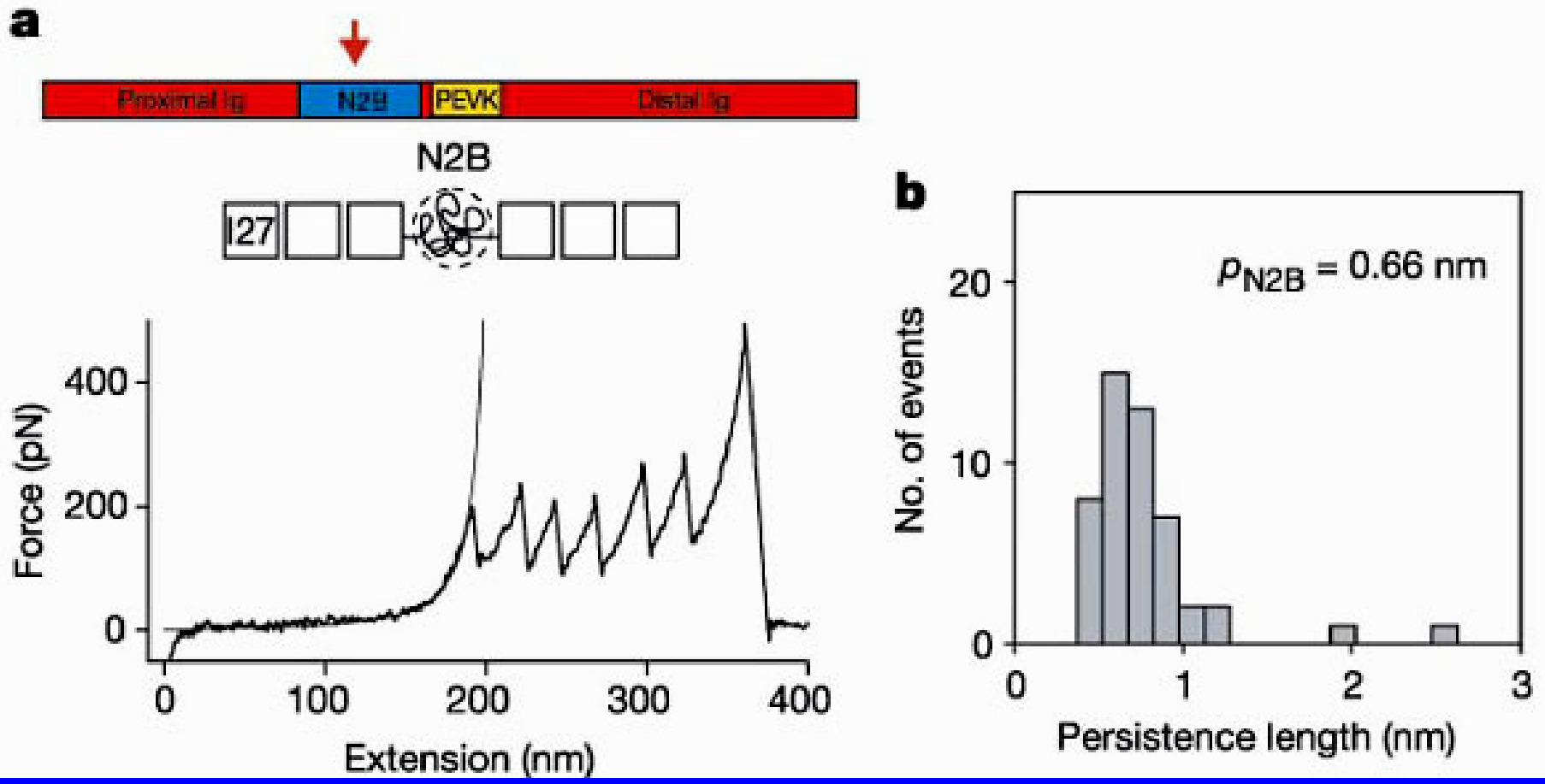
(A) Protein Unfolding



(B) Protein Distortion

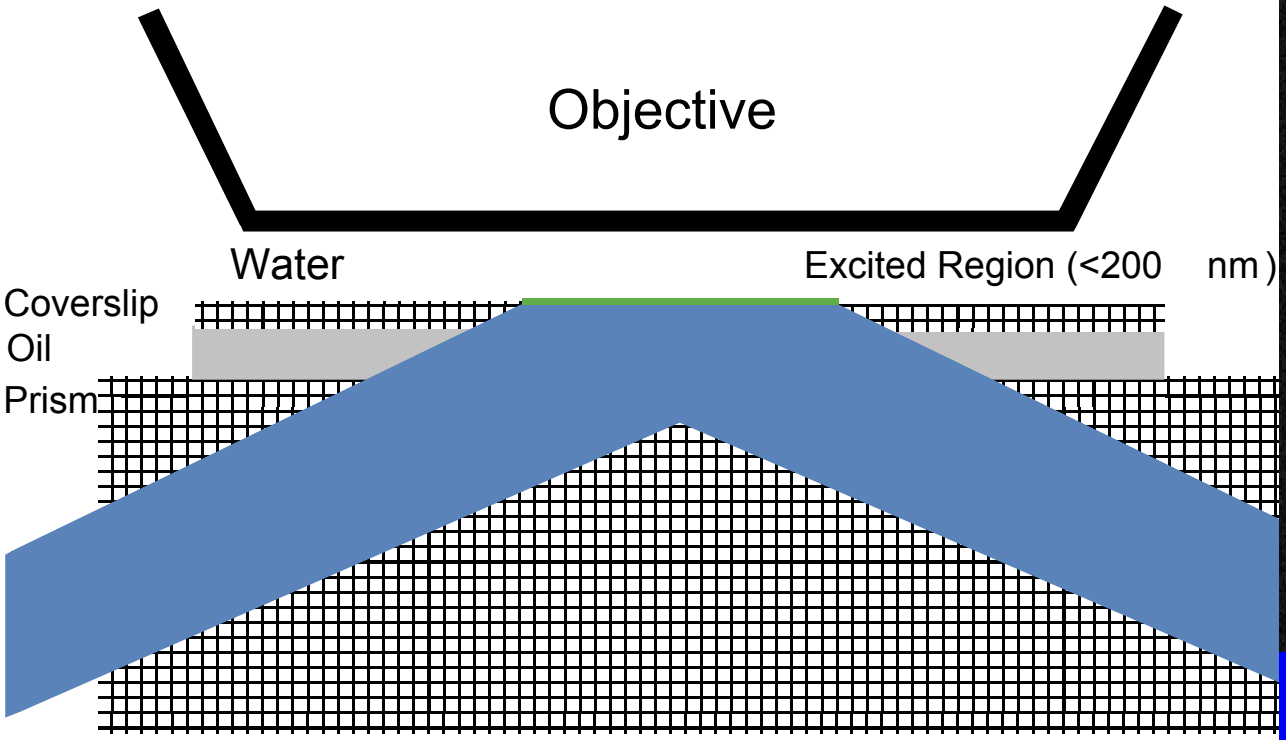


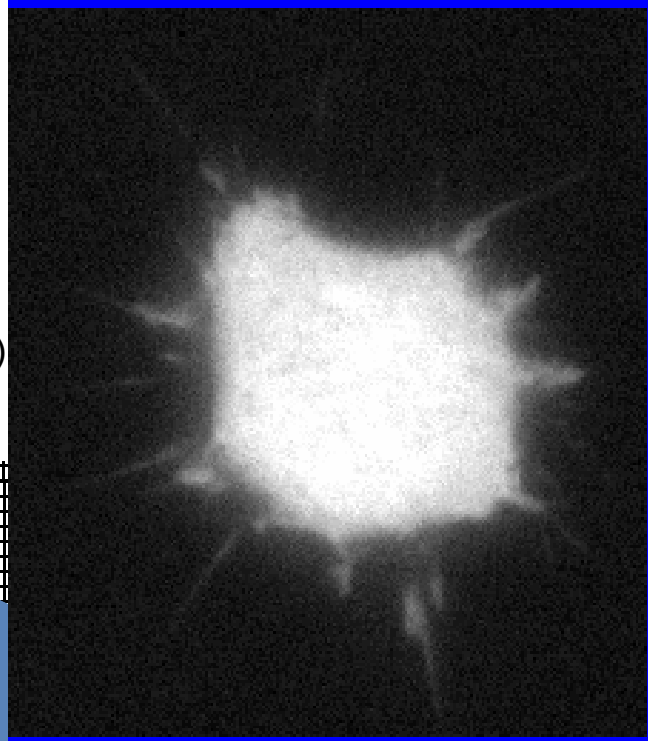
Titin Domains Unfold as Expected for a Force Sensor

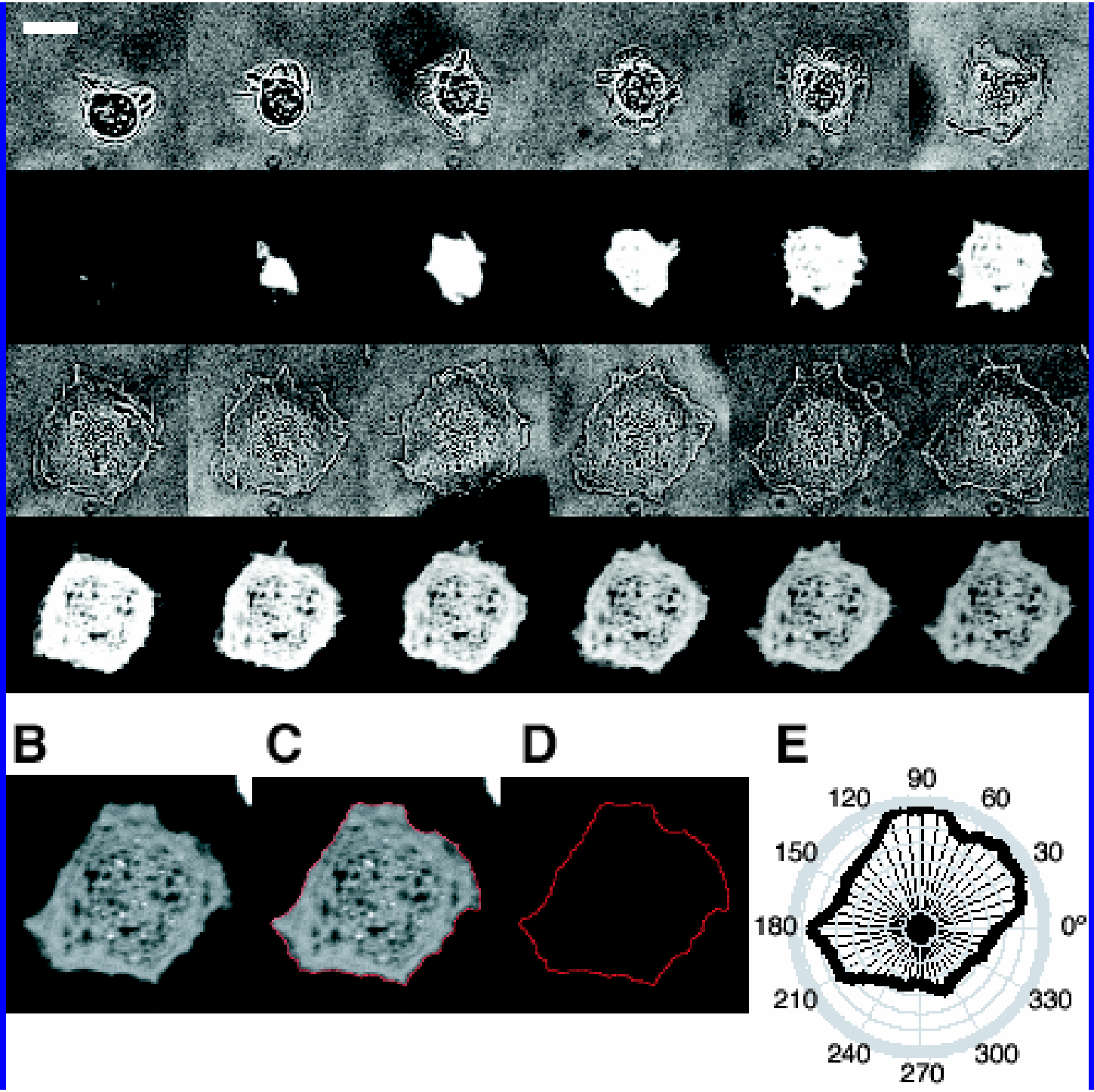


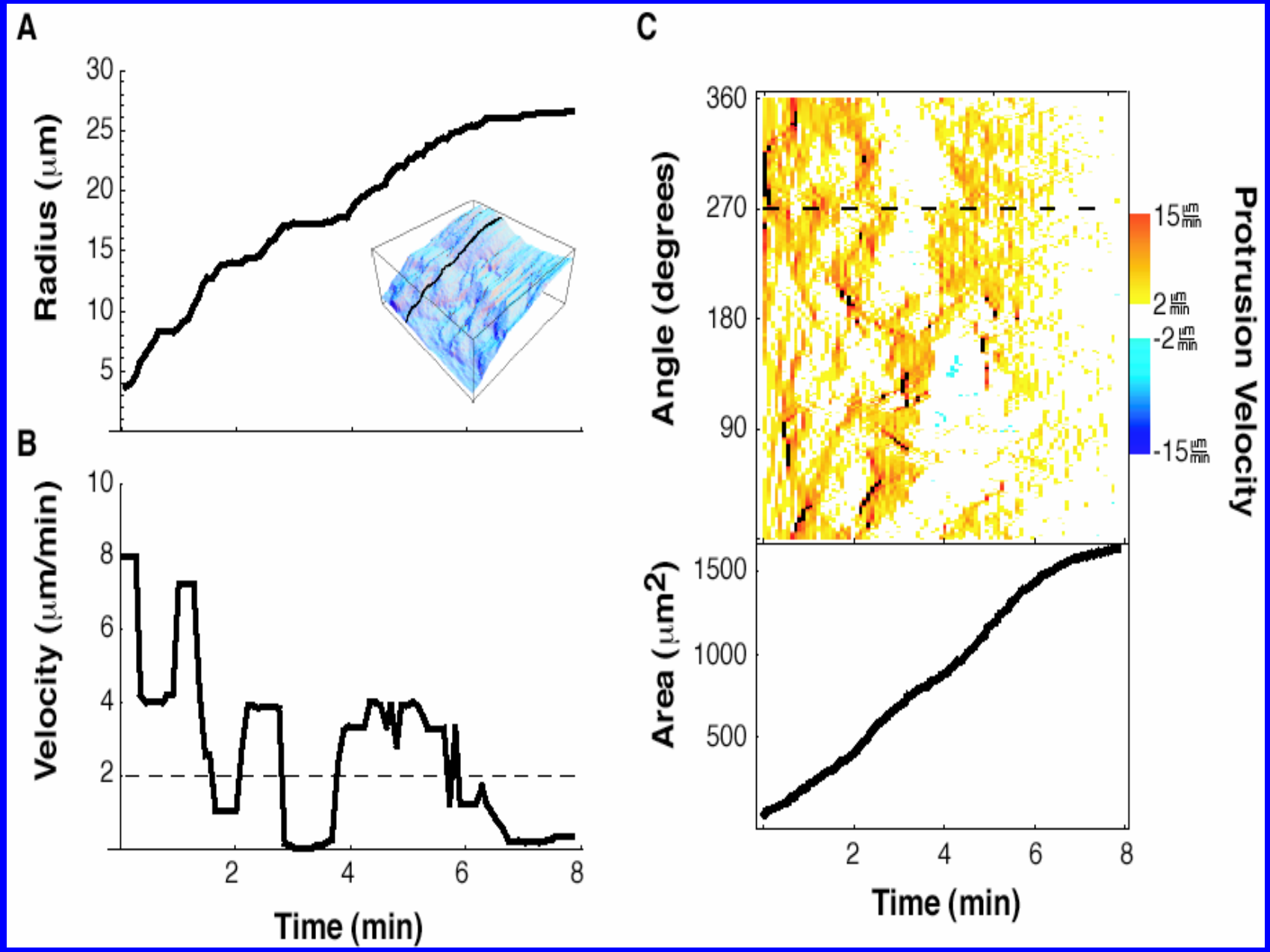
Li et al., (2002) Reverse Engineering of Titin. Nature, 418:998-1002

Total Internal Reflection Fluorescence of Calcein-loaded Cell Spreading

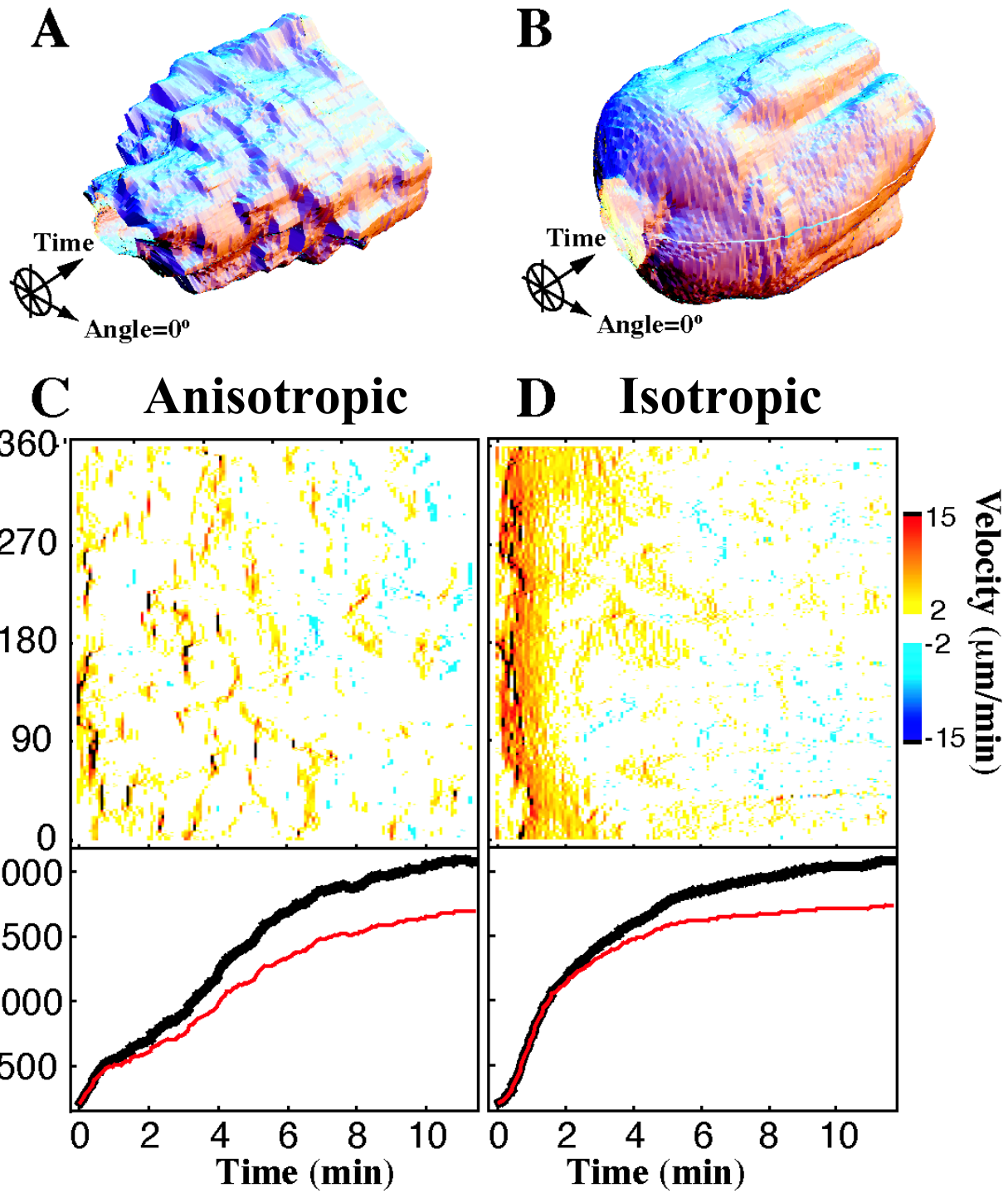




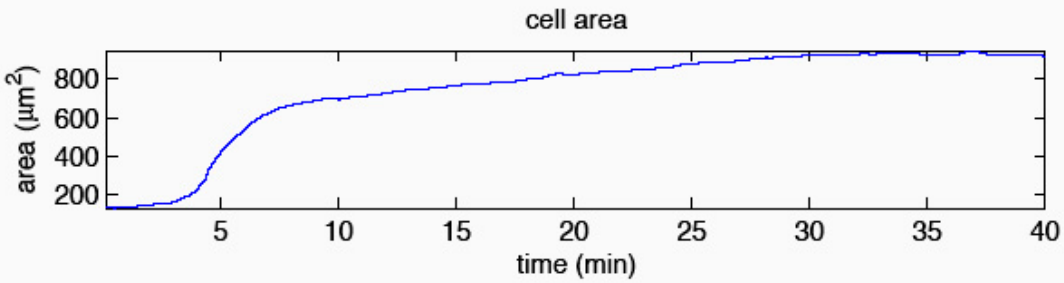

B
C
D
E
90
120
60
150
30
180
0°
210
330
240
300
270

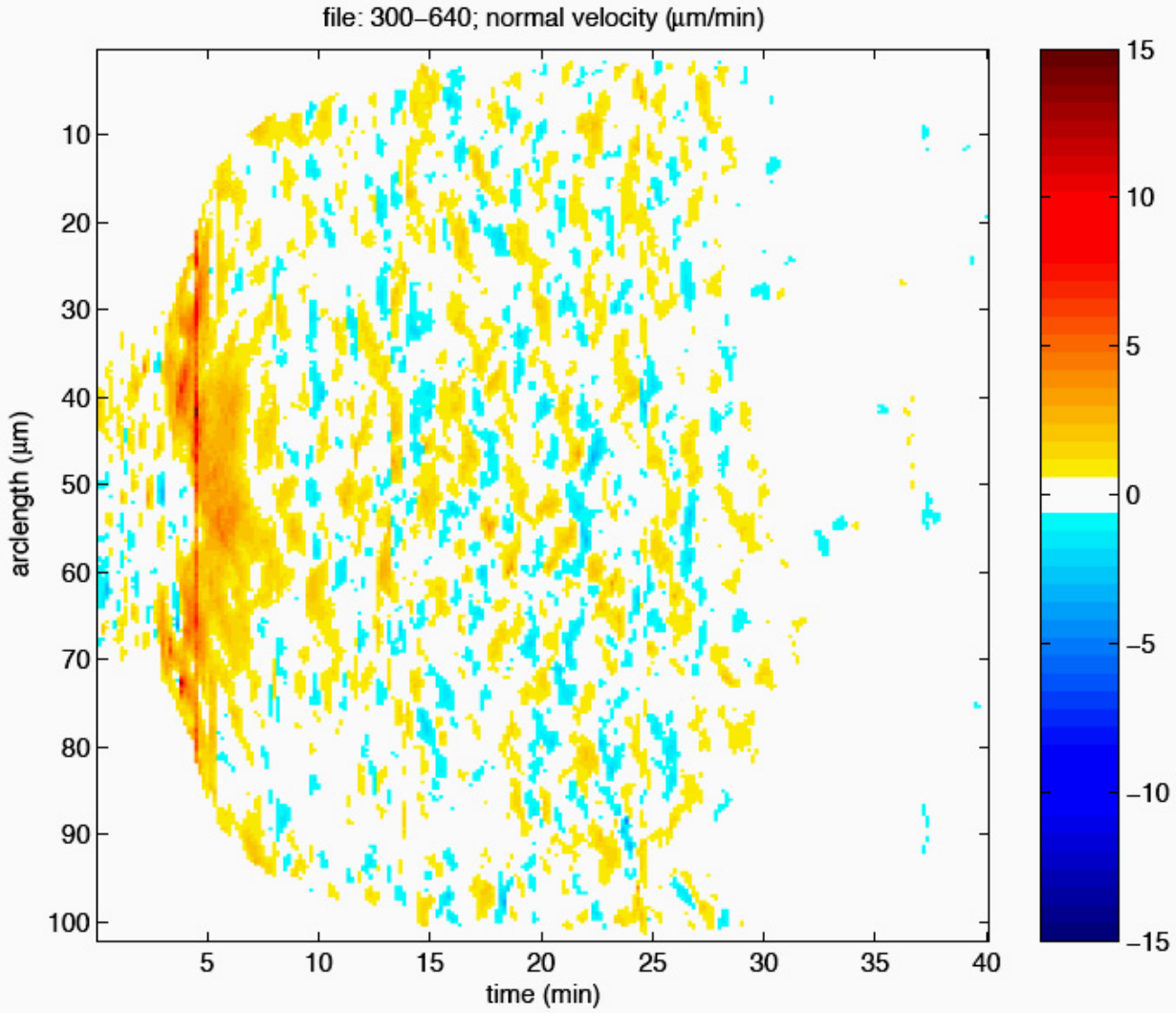


Dubin-Thaler et al., Biophys. J, 86:1794 (2004).


A
Time
Angle=0°
B
Time
Angle=0°
C Anisotropic
D Isotropic
Angle (degrees)
360
270
180
90
0
15
2
-2
-15
Velocity (μm/min)
Area (μm²)
2000
1500
1000
500
0 2 4 6 8 10
Time (min)
0 2 4 6 8 10
Time (min)

MARCKS +/+





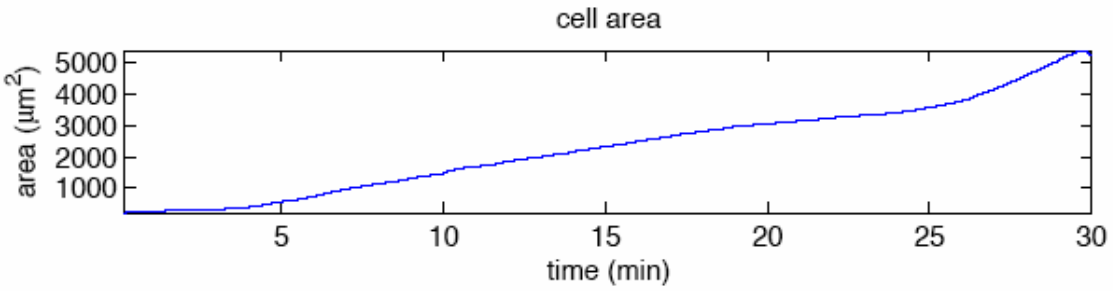
Harry Xenius

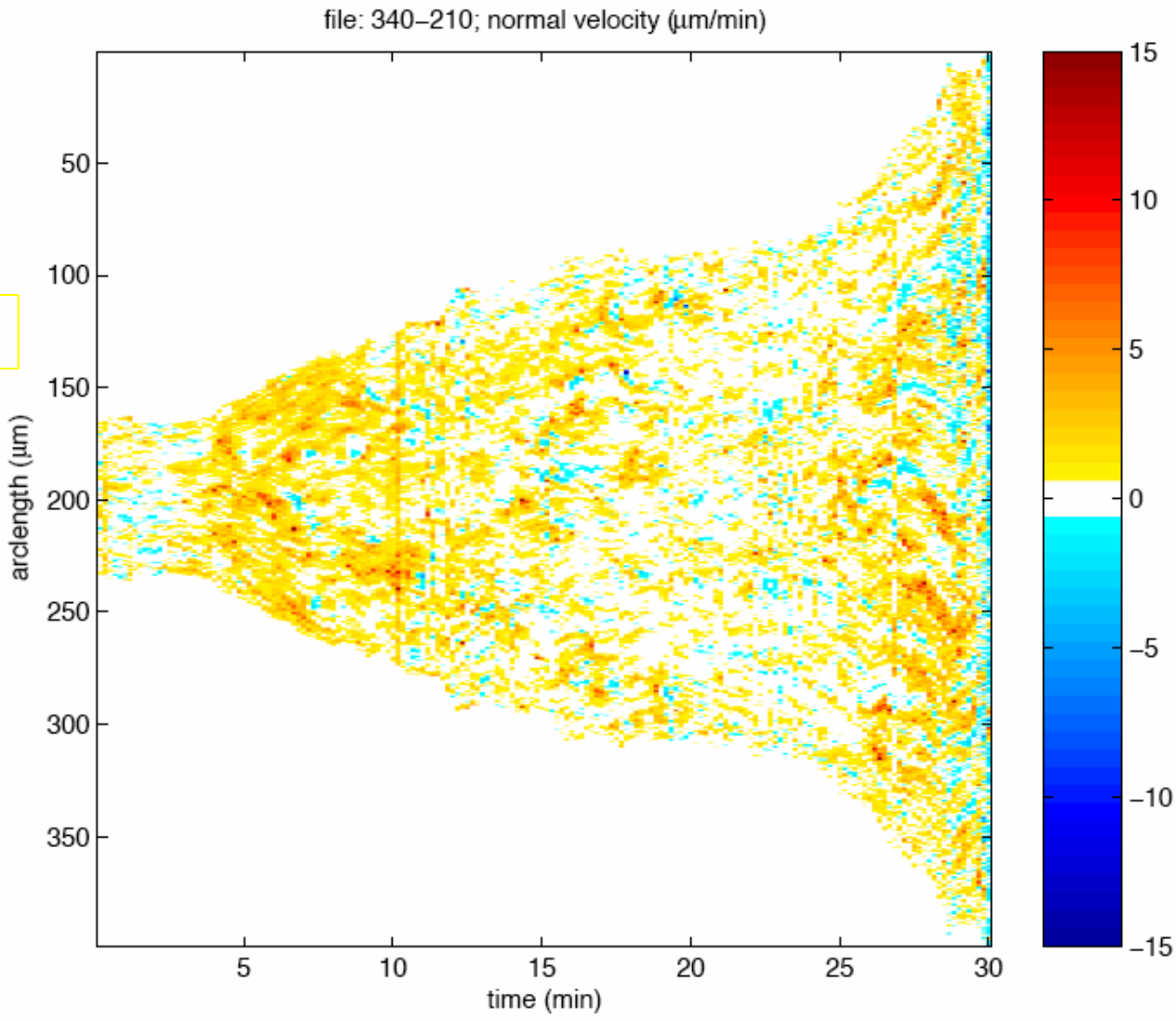
Collaborators

Chris Wiggins

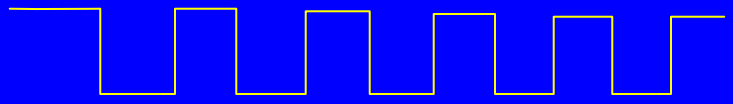
Jake Hofman

MARCKS-/-


cell area
area (μm²)
5000
4000
3000
2000
1000
5
10
15
20
25
30
time (min)

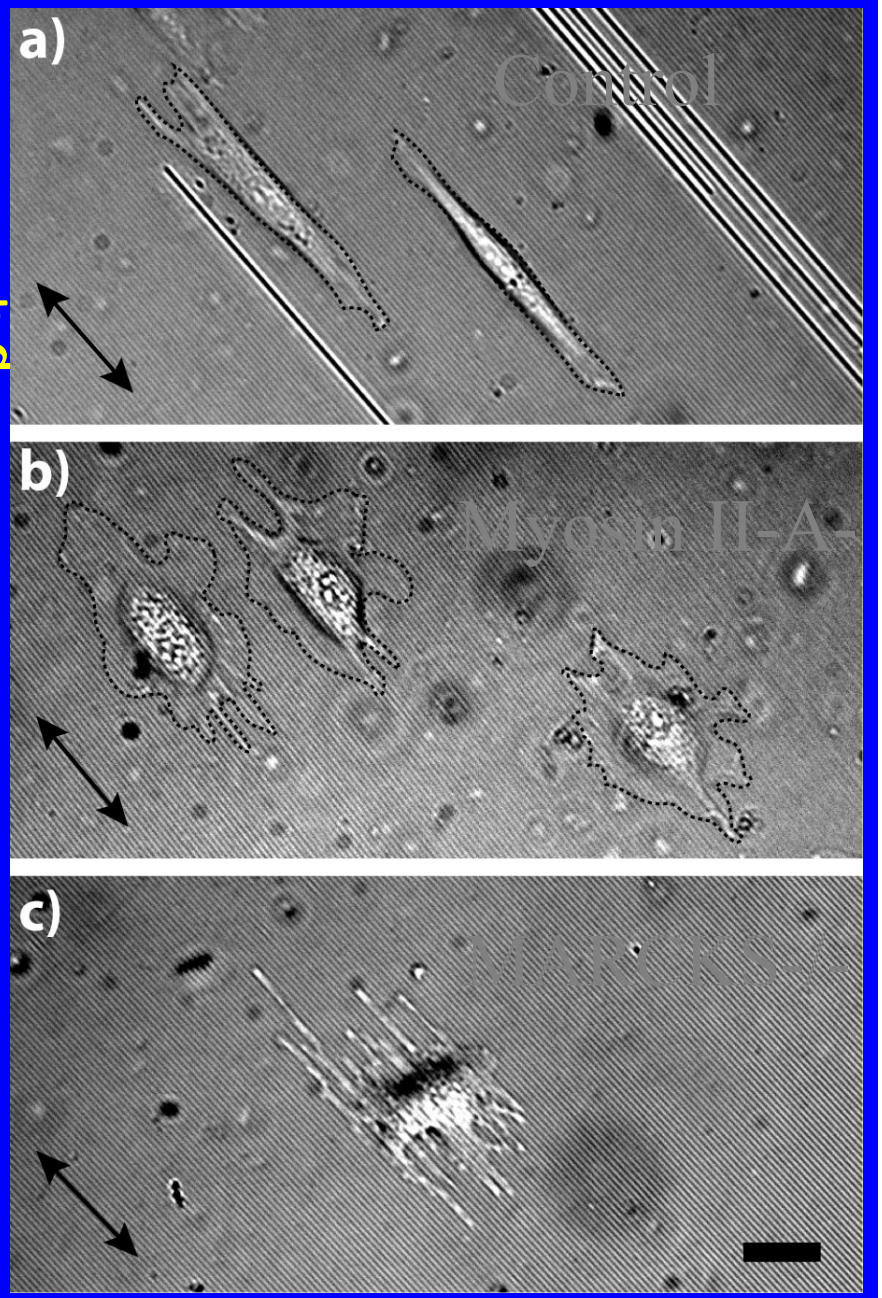

file: 340–210; normal velocity (μm/min)
arclength (μm)
50
100
150
200
250
300
350
5
10
15
20
25
30
time (min)
15
10
5
0
–5
–10
–15

Regulation of Morphology by Substrate Engineering

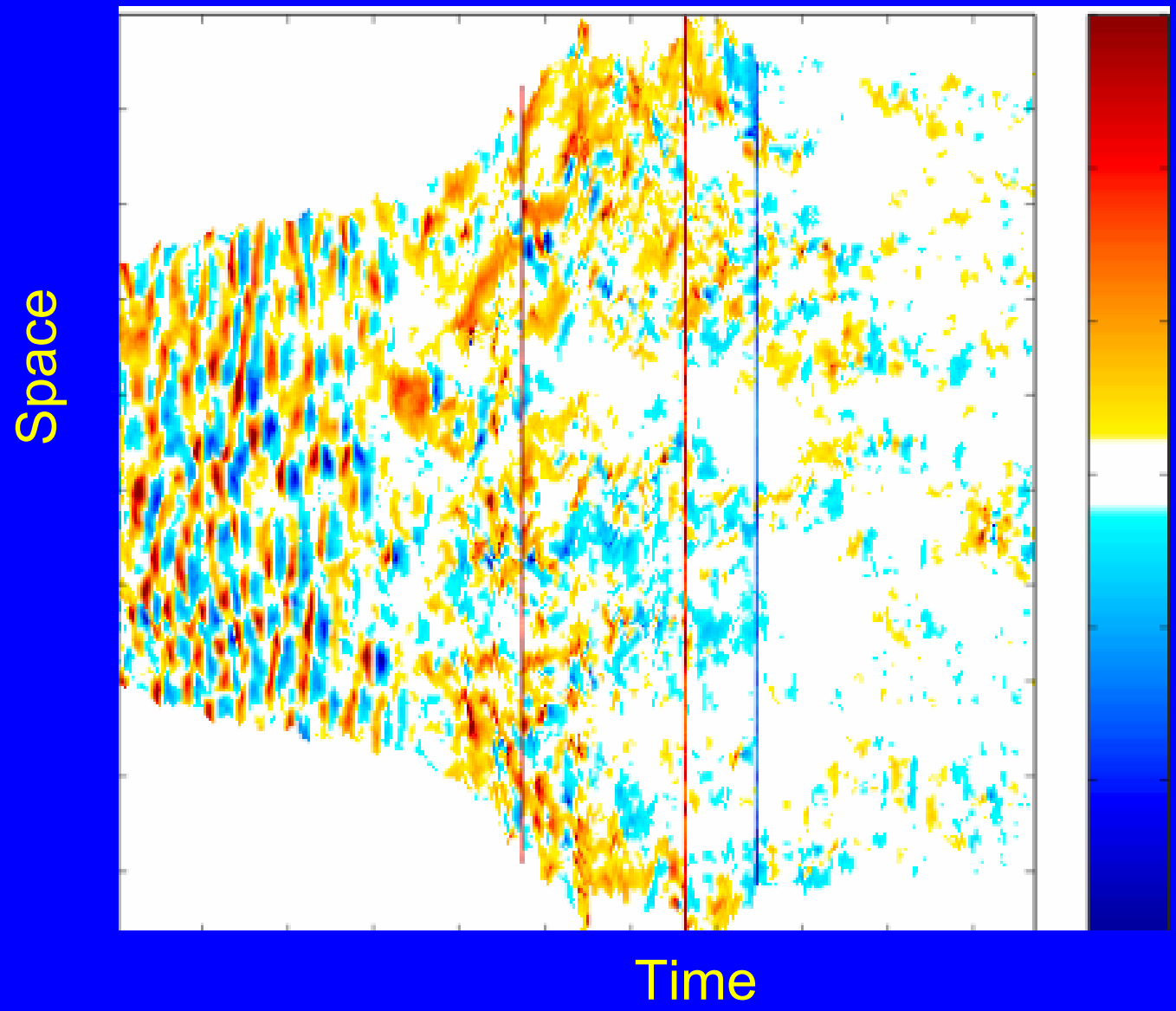


Substrates have 1μm ridges separated by 1 μm

Understanding the biochemical and biophysical basis of cell morphology



Evidence for Spatial and Temporal Order



Cross-Correlation Analyses in Space and Time

$v(t,s) \equiv$ membrane velocity matrix - average velocity

$T \equiv$ total time of $v(t,s)$ $\quad$ $S \equiv$ total arclength of $v(t,s)$

$\Delta t \equiv$ lag in time $\quad$ $\Delta t \equiv$ lag in space

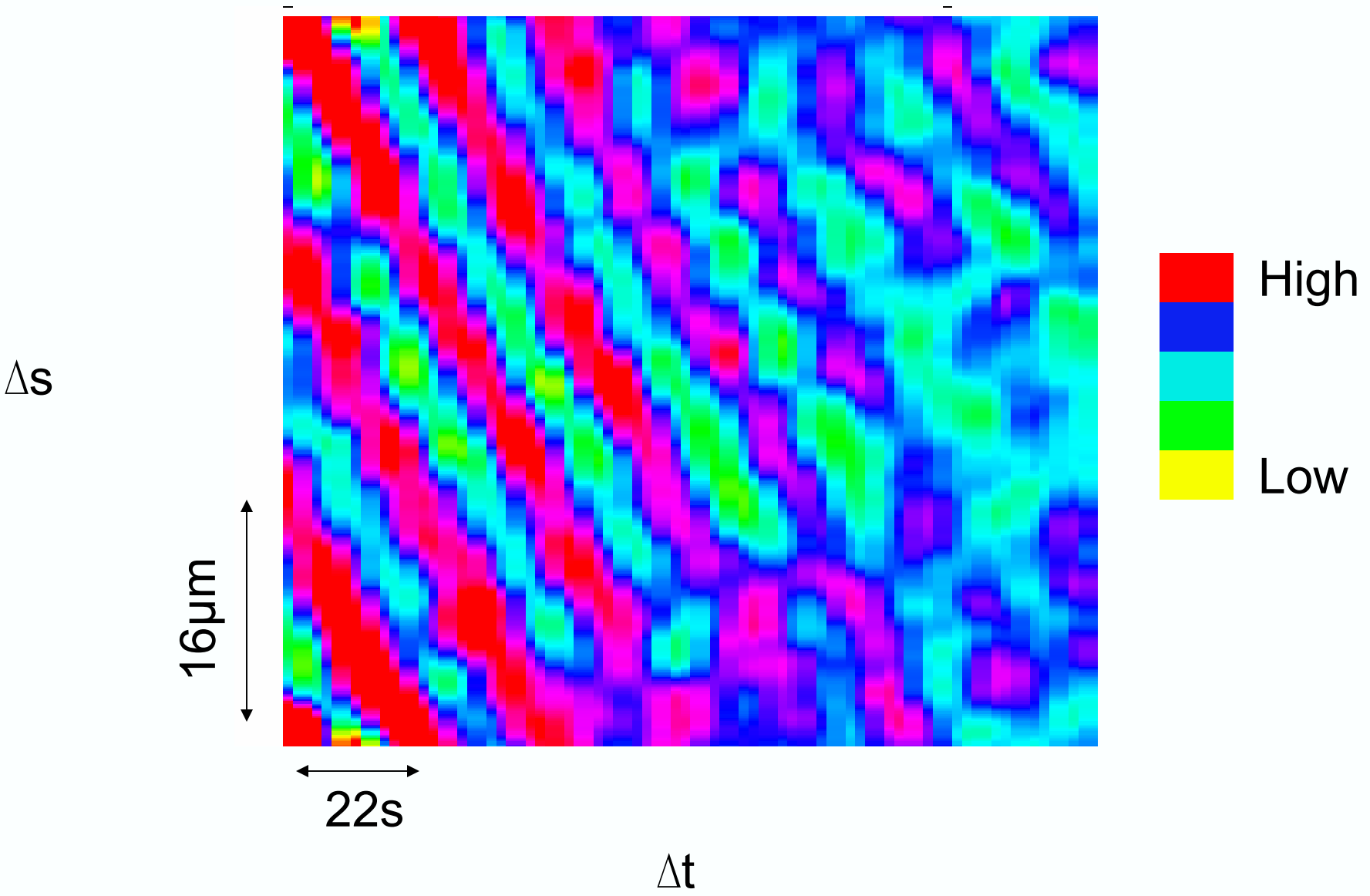
Correlation for a given point:

$$\text{correlation}(\Delta t, \Delta s) = v(i,j) * v(i+\Delta t, j+\Delta s)$$

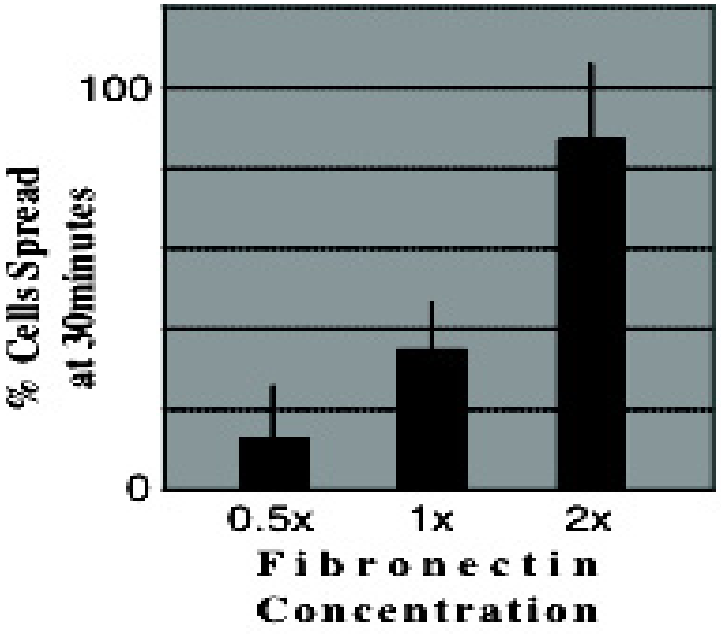
Correlation averaged over all points:

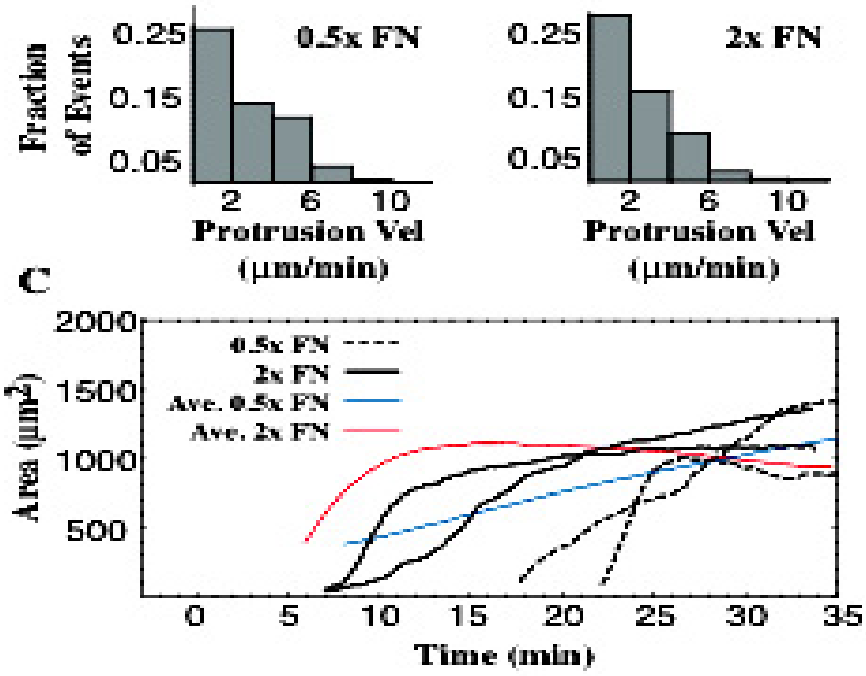
$$\text{correlation}(\Delta t, \Delta s) = \frac{\sum_{i=1}^{T-\Delta t} \sum_{j=1}^{S} v(i,j) * v(i+\Delta t, \text{mod}(j+\Delta s, S))}{(T-\Delta t) * S}$$

Cross-Correlation Reveals Repeats at 16 μm and 22s



FN Concentration Affects Initiation, Not Rate of Spreading

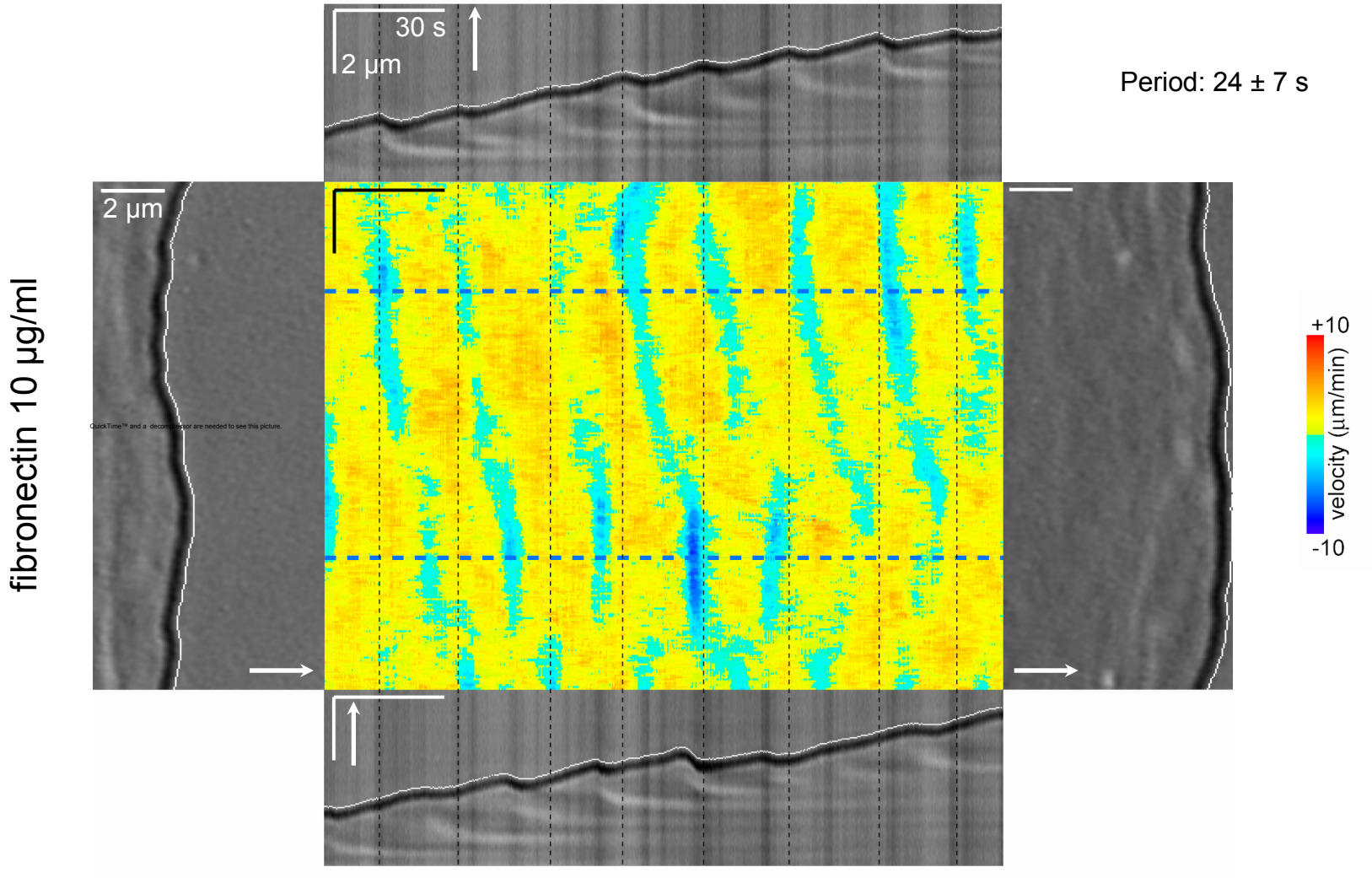




Isotropic Spreading with GFP-α-actinin

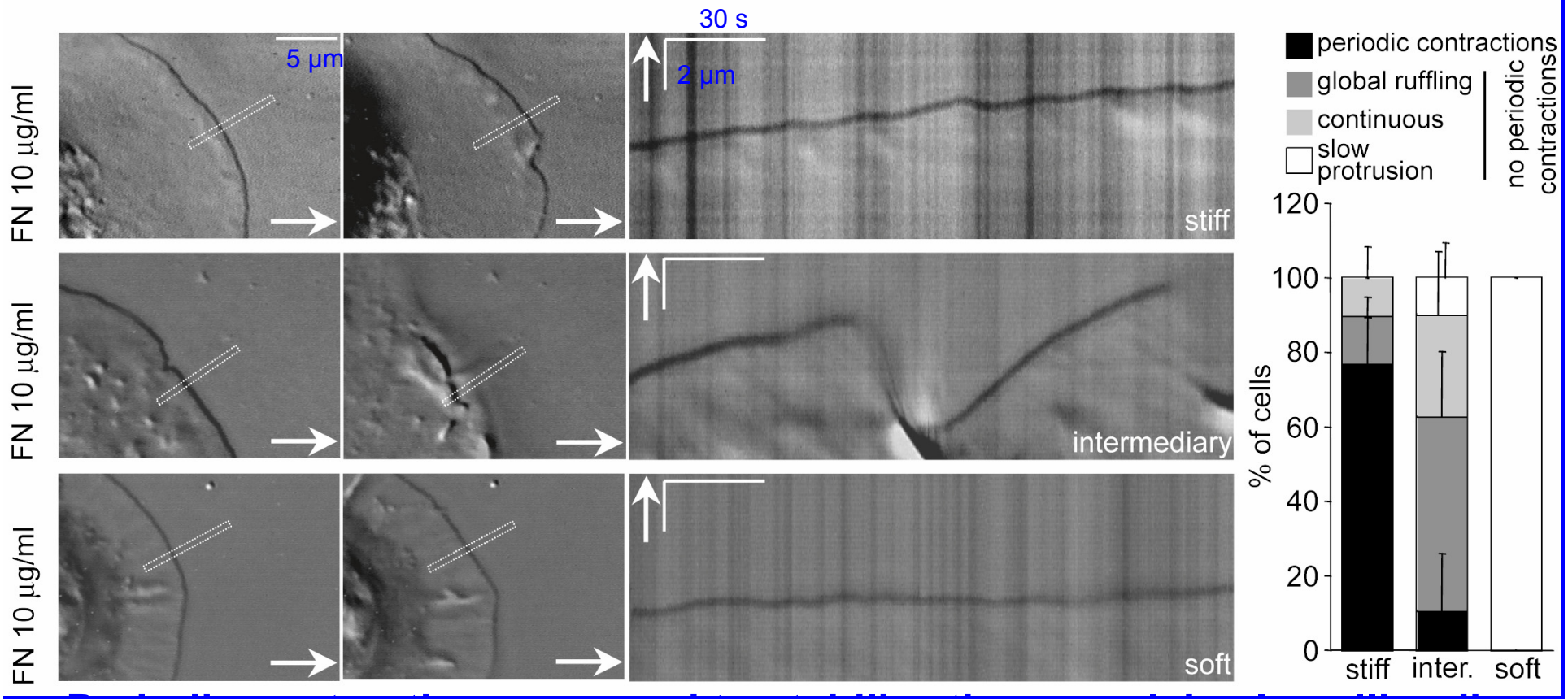
QuickTime™ and a Video decompressor are needed to see this picture.

Local periodic retractions of the cell edge during lamellipodial extension

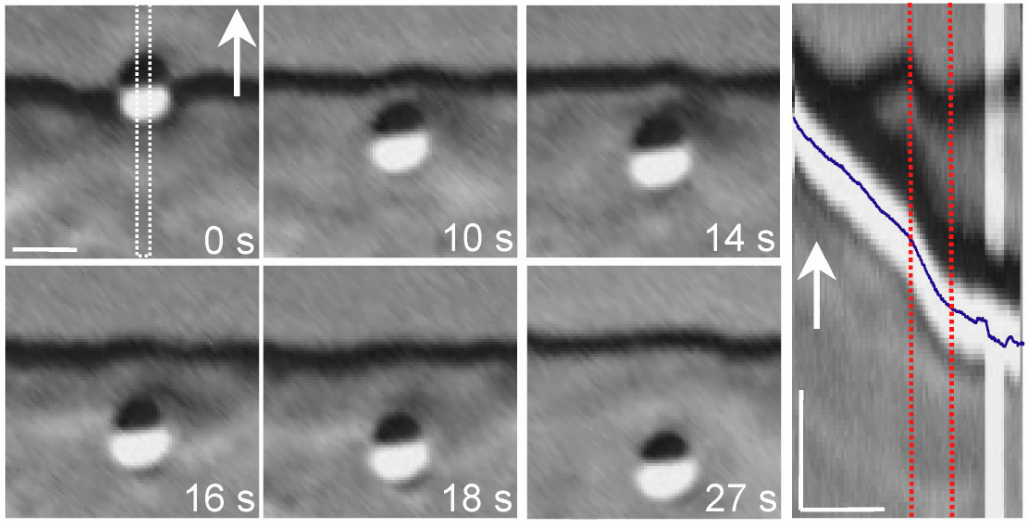


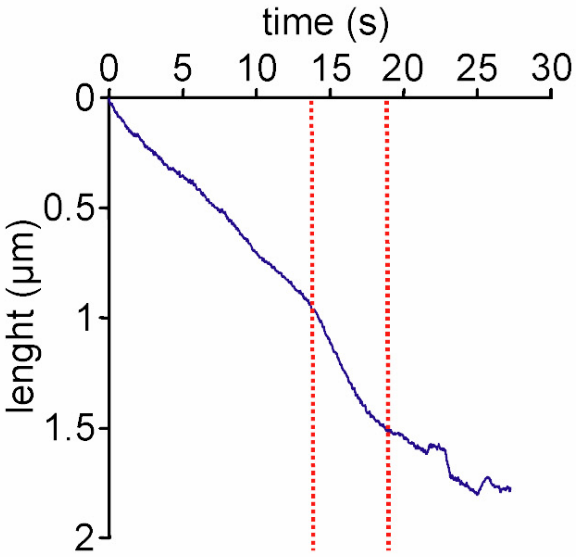
Giannone et al., (2004) Cell. 116:431-43.

Periodic contractions depend on a stiff substrate



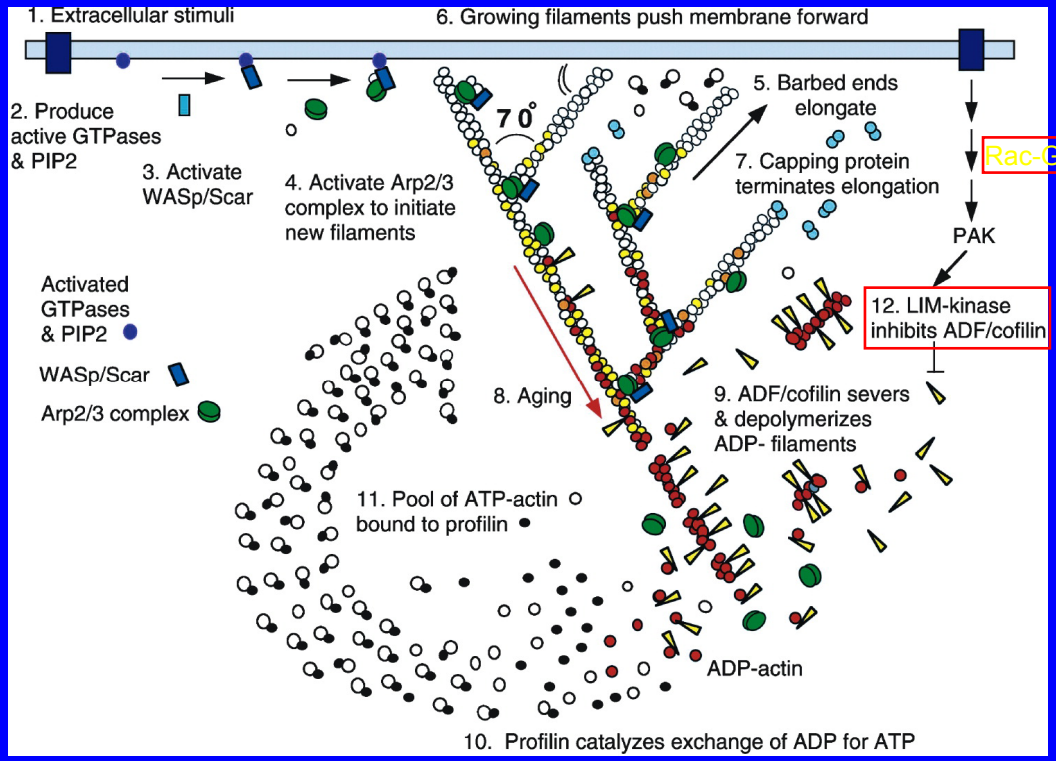
Periodic Retractions Are Periodic Contractions of the Lamellipodia

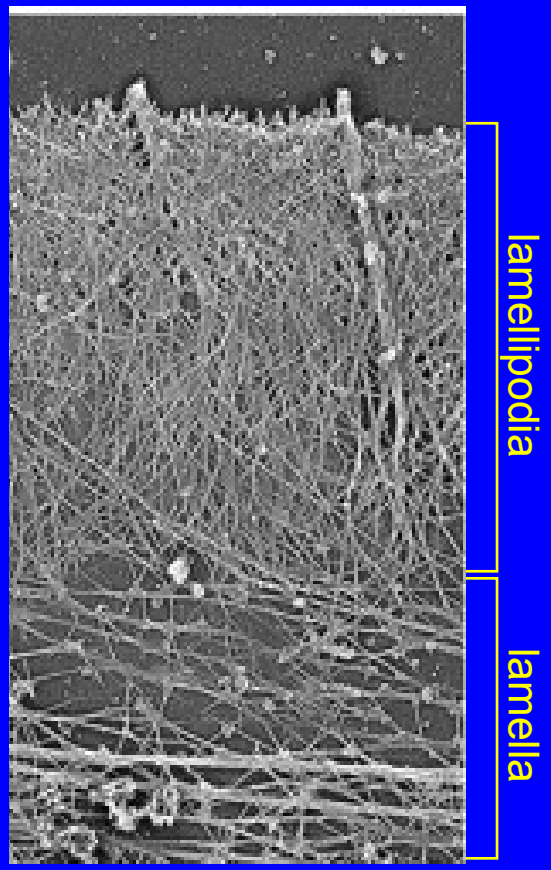




QuickTime™ and a decompressor are needed to see this picture.

Lamellipodial extension driven by actin assembly





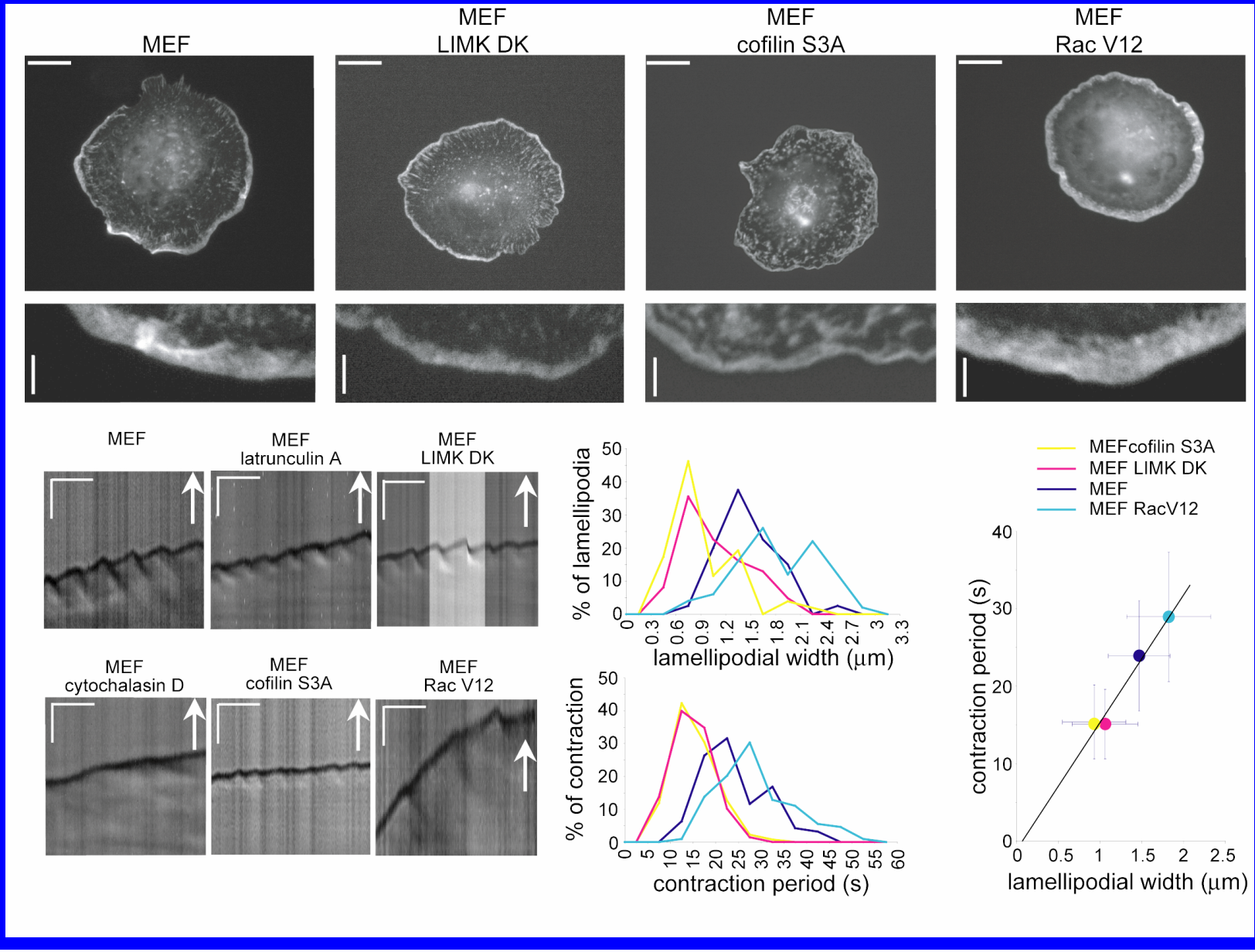
Actin plolymerization (front) and depolymarization(rear)

define the lamellipodial width

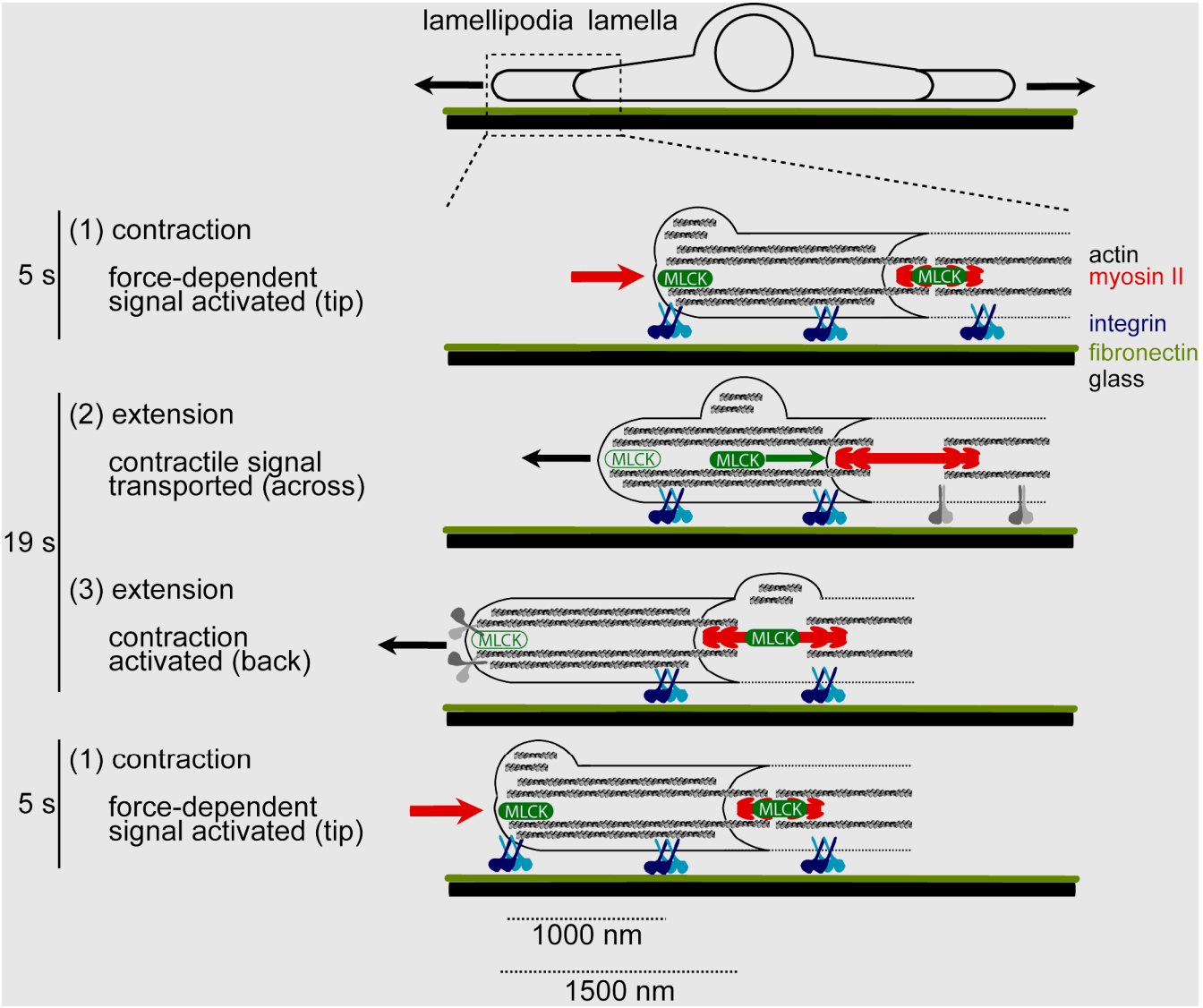
Forces generated by Myosins?

Cell spreading and migration require myosin activity

Period Set by Width of Actin Meshwork



Model of signaling by cytoskeletal transport

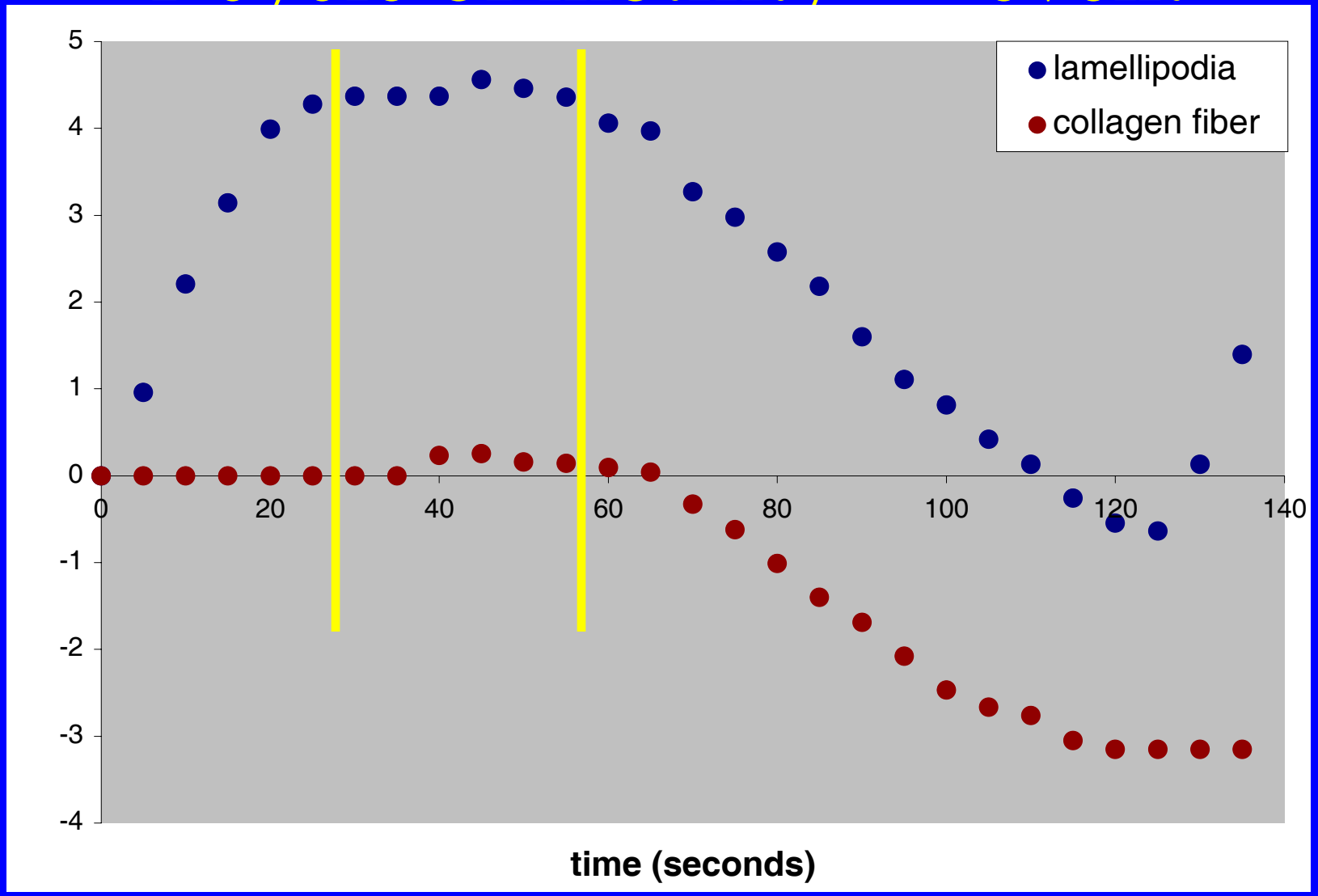


Hand-Over-Hand Movement of Collagen Fibers by Myosin II-B

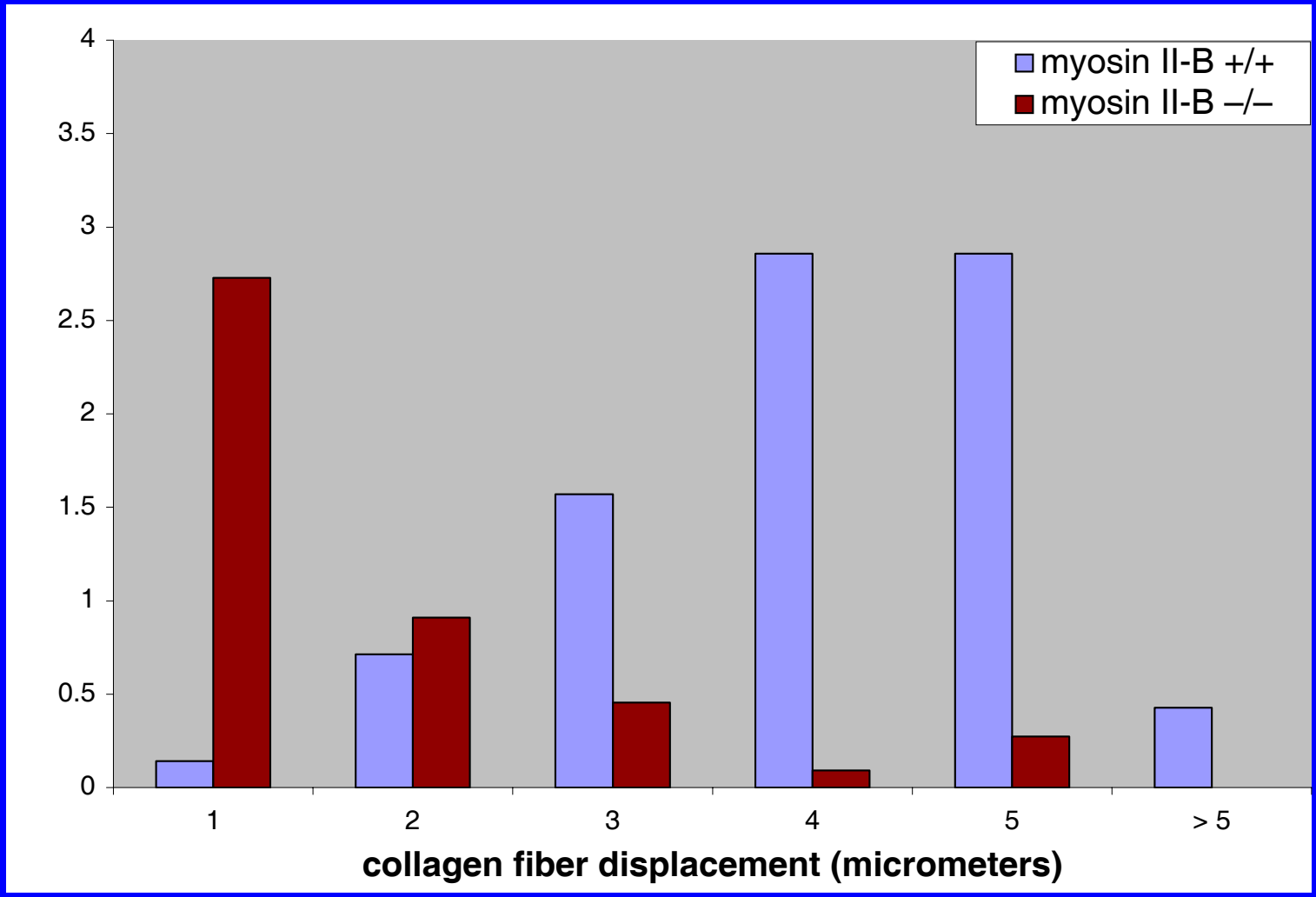
QuickTime™ and a Video decompressor are needed to see this picture.

Meshel, Wei, Adelstein & Sheetz, 2005 Nat. Cell Biol. 7:157

1 cycle of motility = "event"



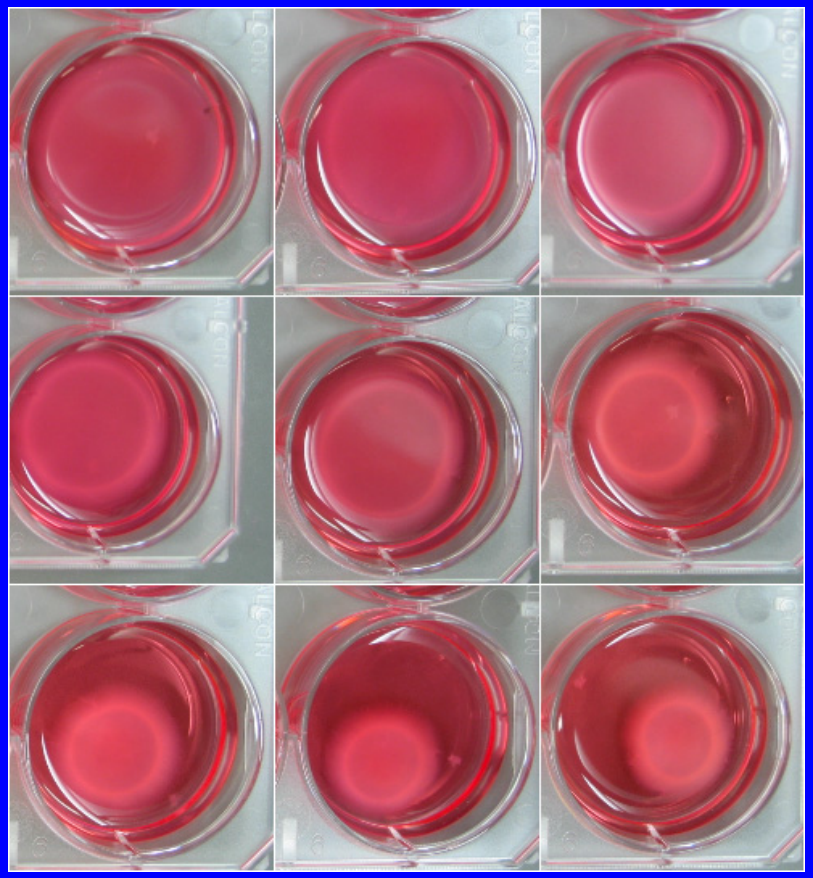
II-B –/– cells have slower, shorter events



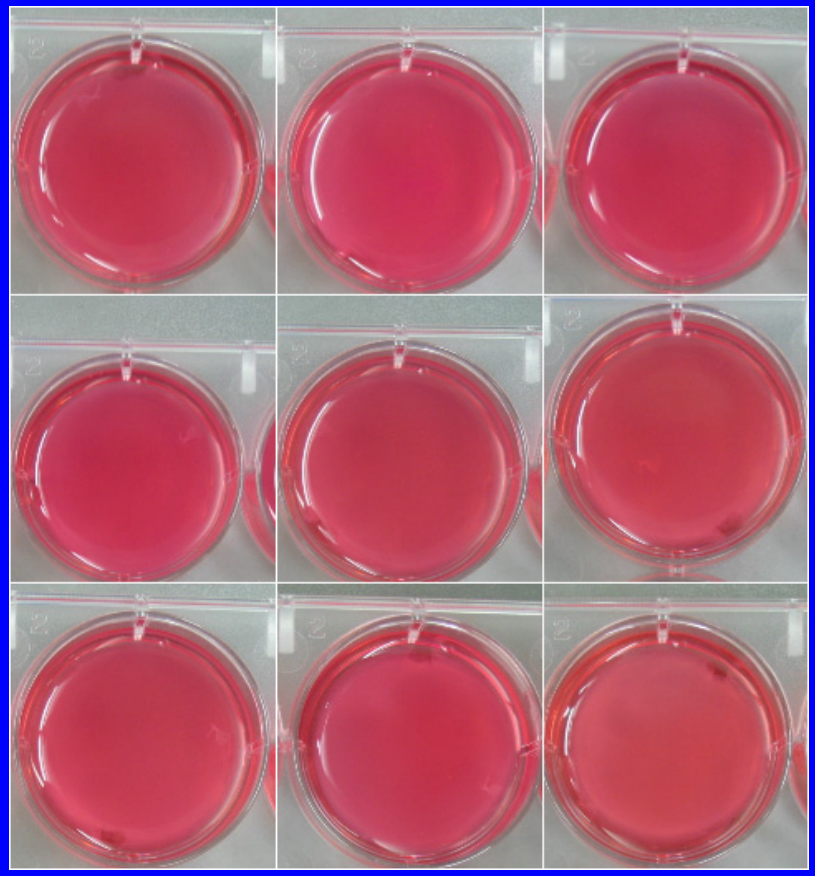
*retransfection of GFP-NMHC II-B restores function while GFP-II-A does not...

Myosin II-B is required for 3-D collagen gel contraction

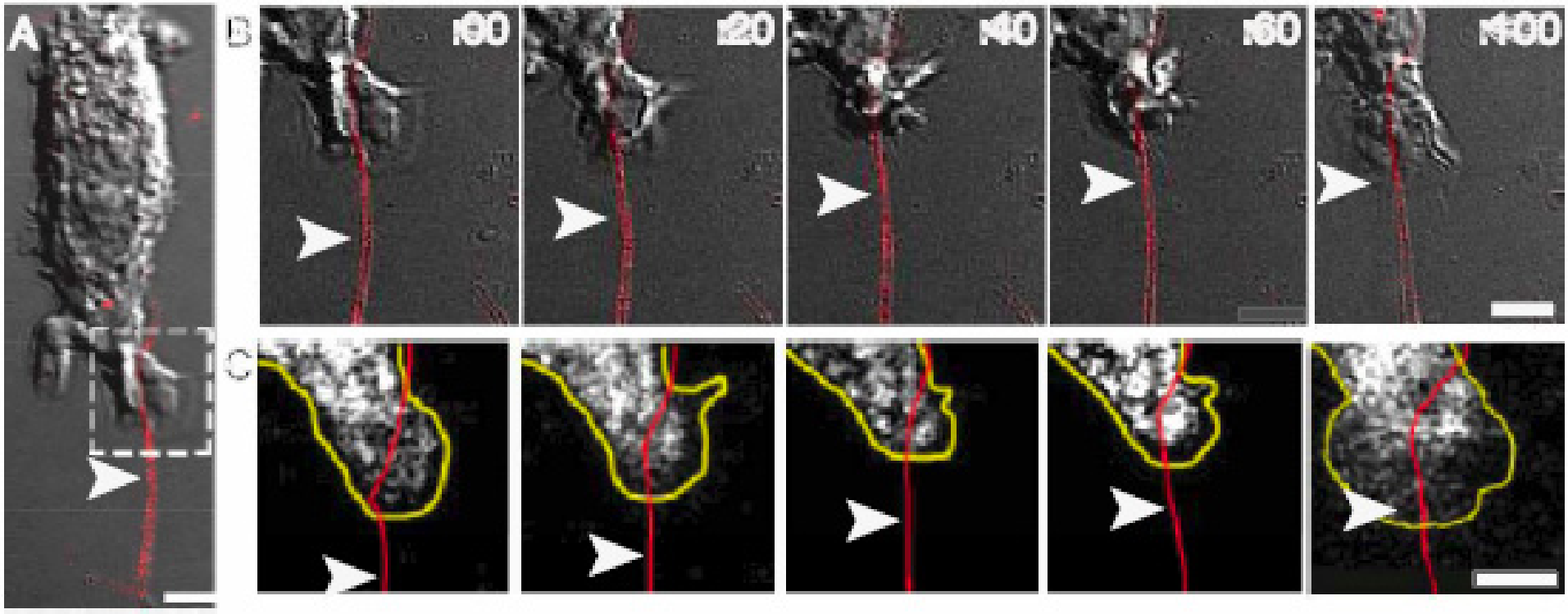
wild type



myosin II-B -/-

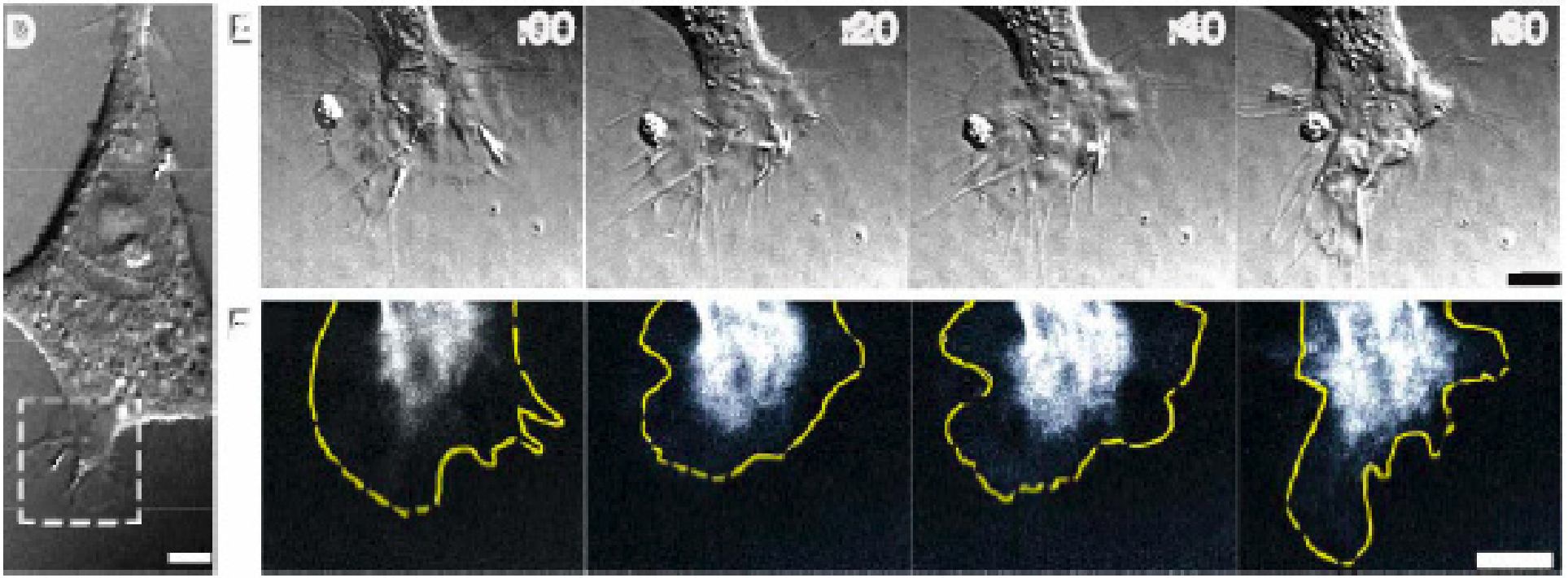


Myosin II-B Assembles in Lamellipodia & Moves With Fiber



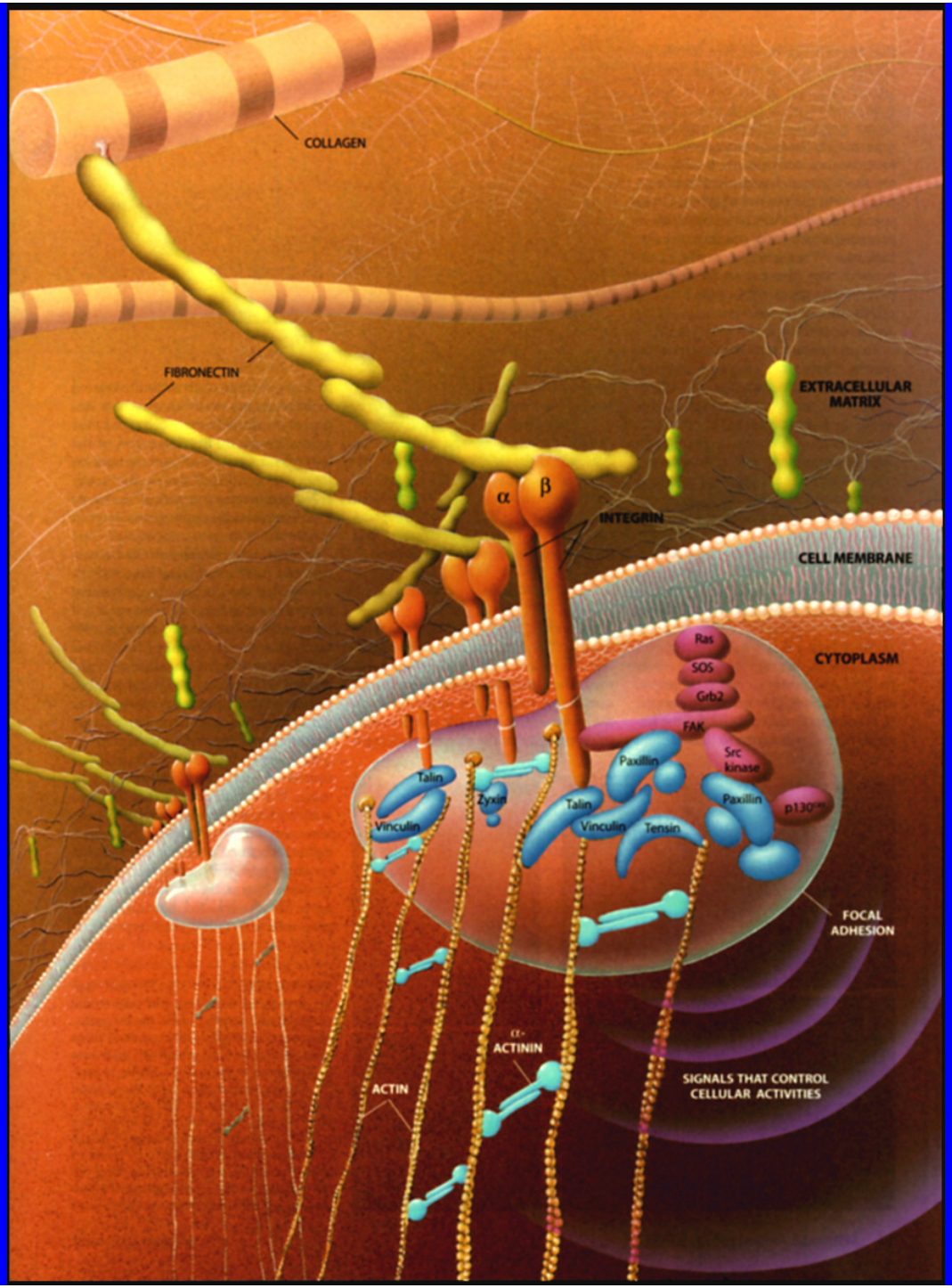
Meshel, Wei, Adelstein & Sheetz, 2005 Nat. Cell Biol. 7:157

Myosin II-B Is Not in Lamellipodia on 2-D Collagen



General Lessons for Robust Functions at Submicron Level

- Compartmentalization and Modular design
- Spatial Ordering of Multiple sites for Specificity
- Functional Activity Turns Off Automatically
- Multiple Activity Cycles for Important Functions
- Many Low Fidelity Steps for High Fidelity Process



Horwitz, Scientific American

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