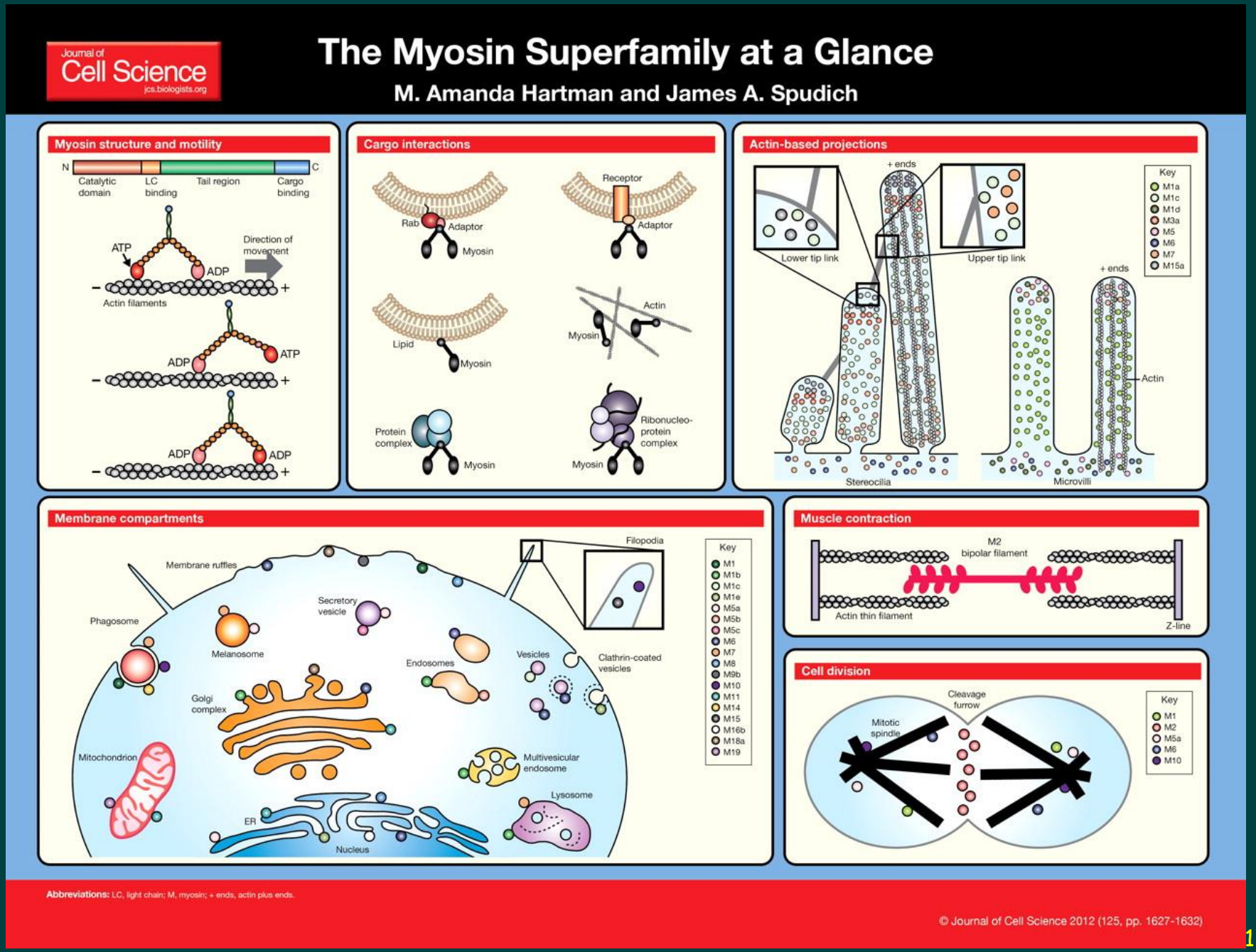
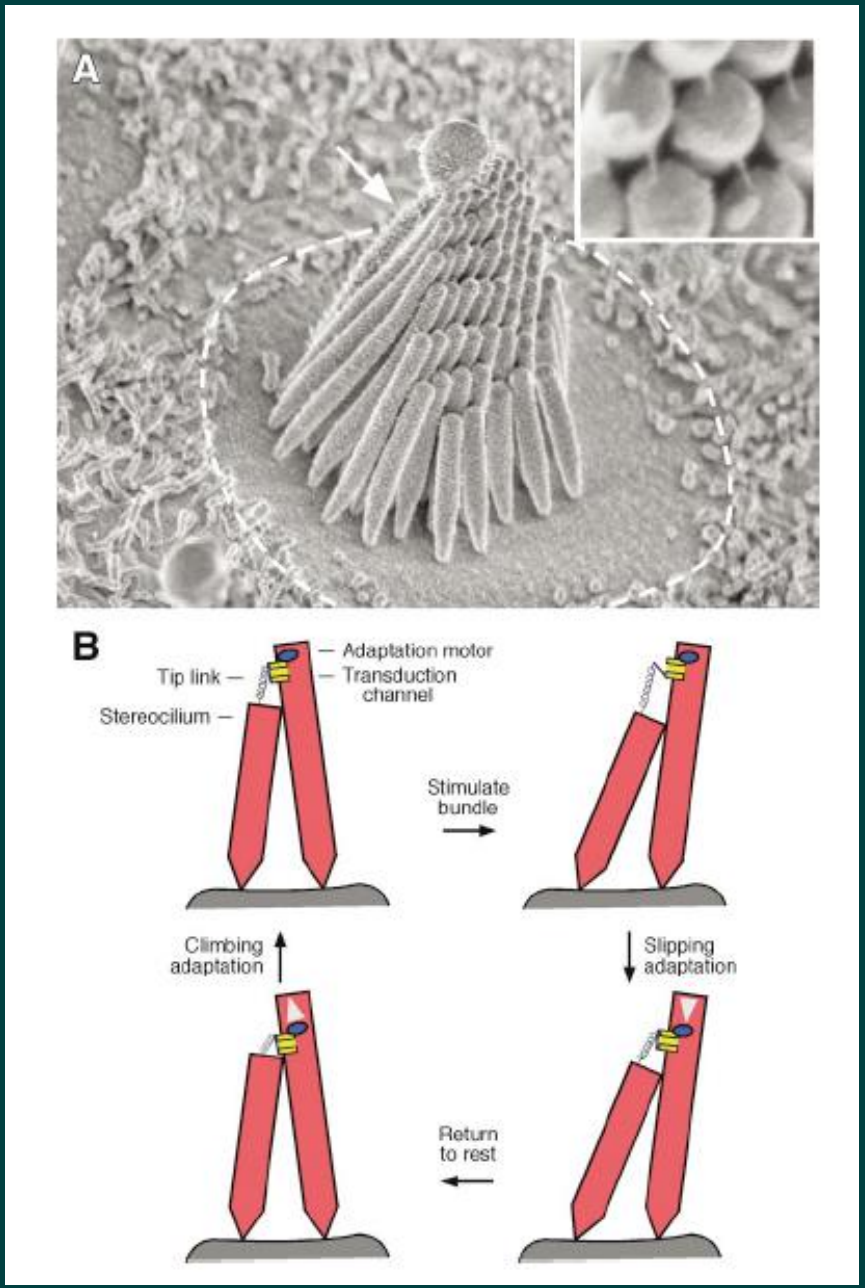
last time: different myosins and their functions



Adaptation motor



myosin II in the muscle

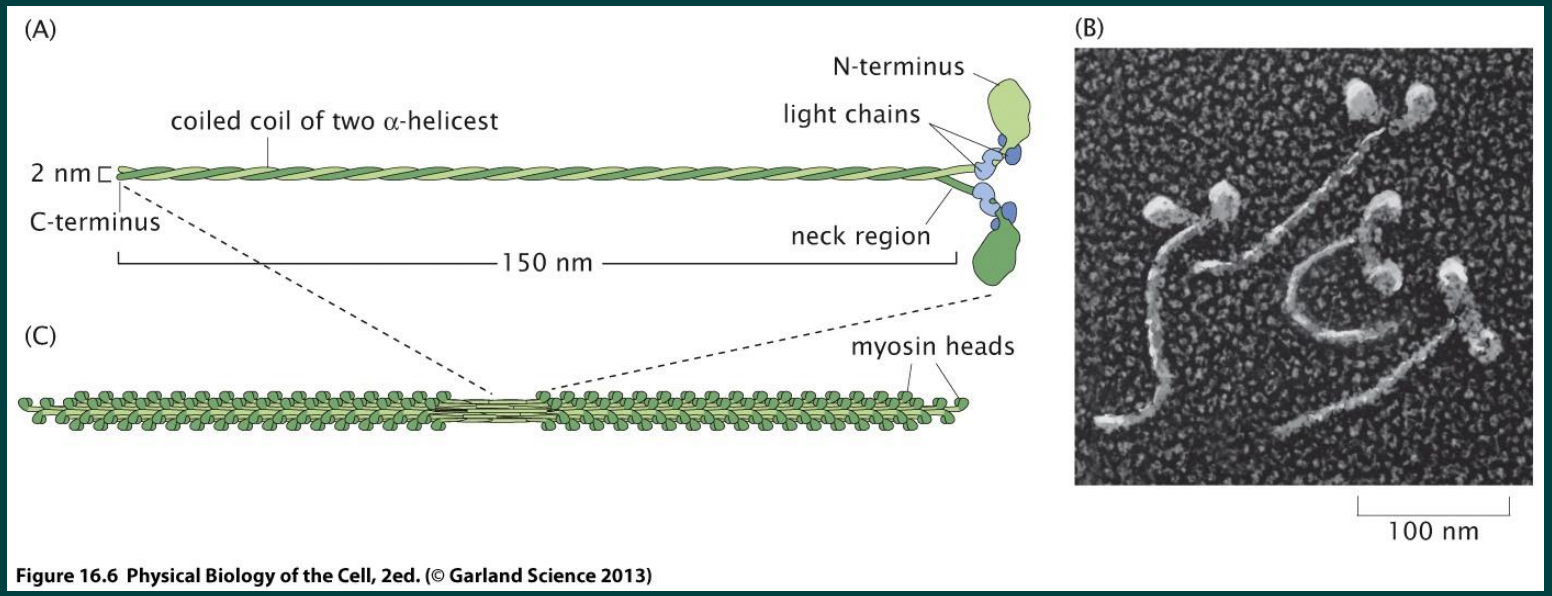


Figure 16.6 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

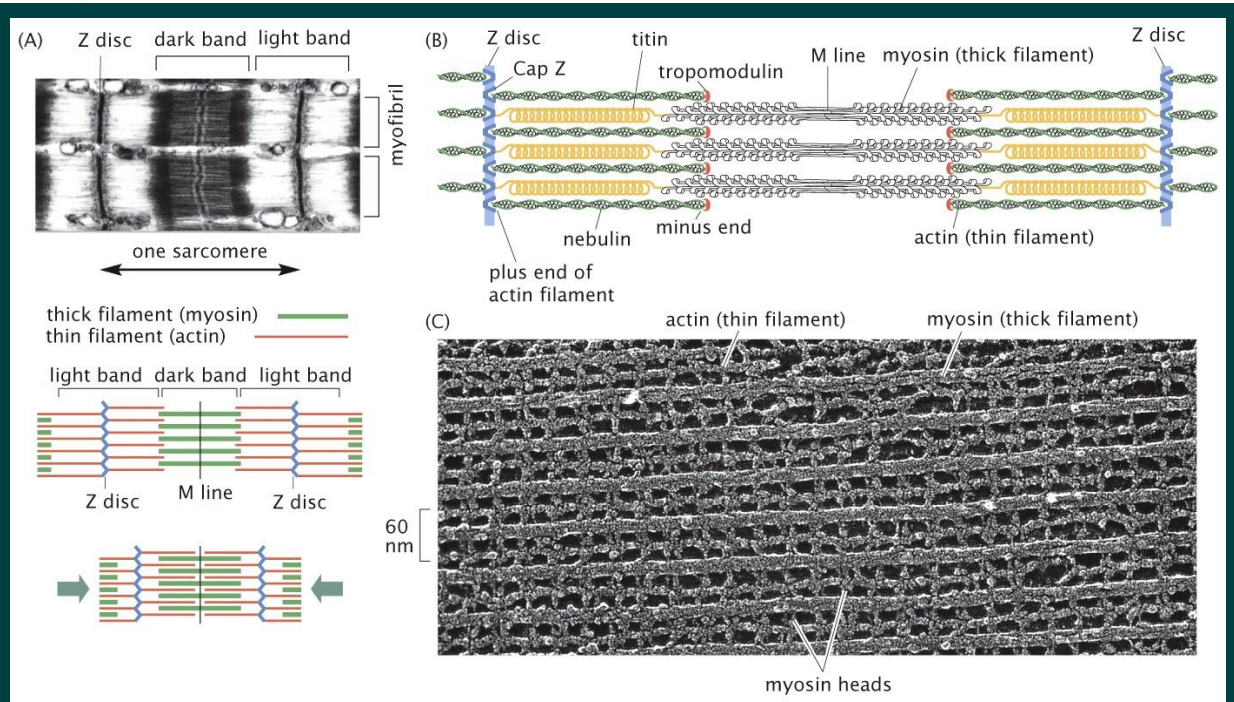
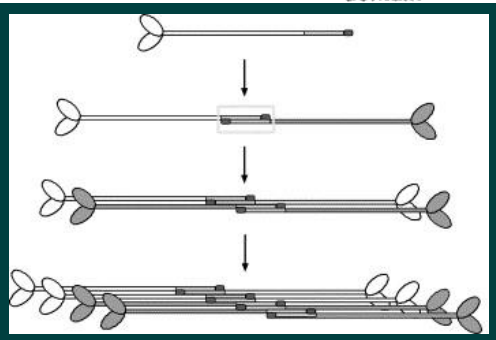
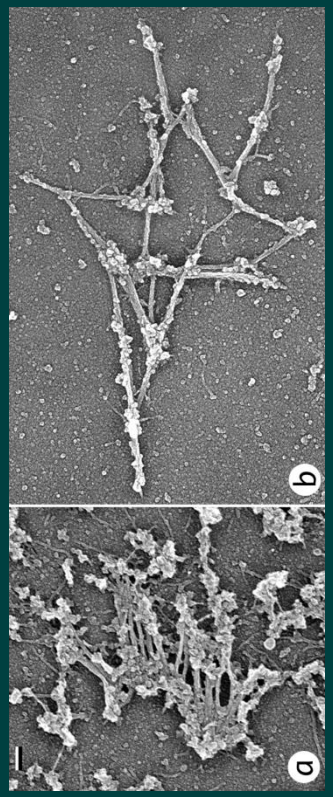
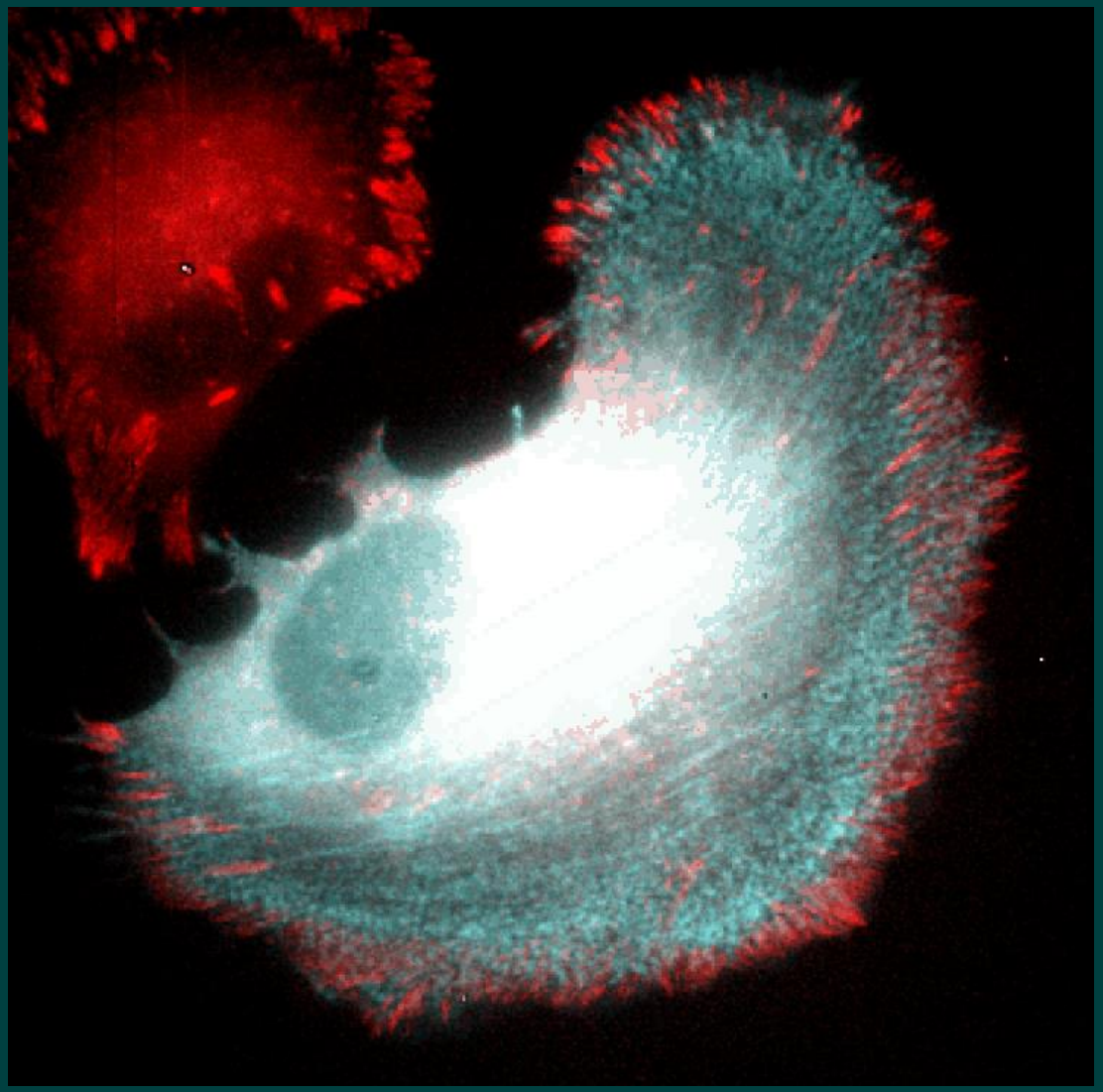


Figure 16.7 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Myosin II in non-muscle cells

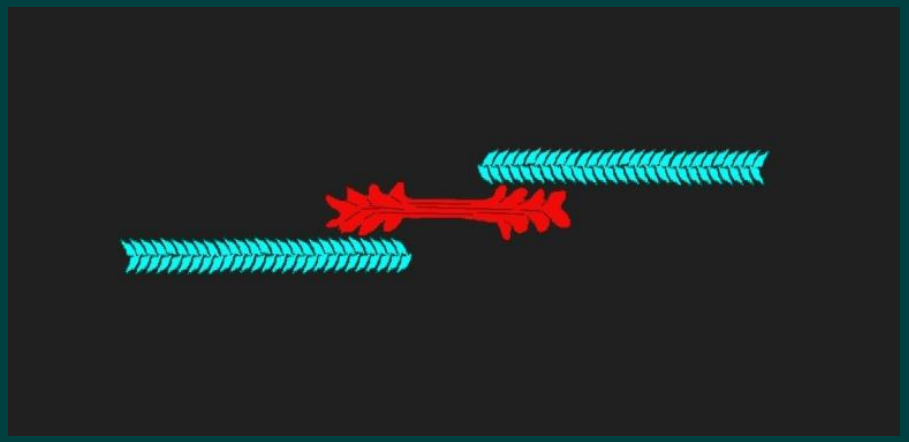






Verkhovsky et al., J. Cell Biol., 1995

proposed role for myosin II in crawling cell motility



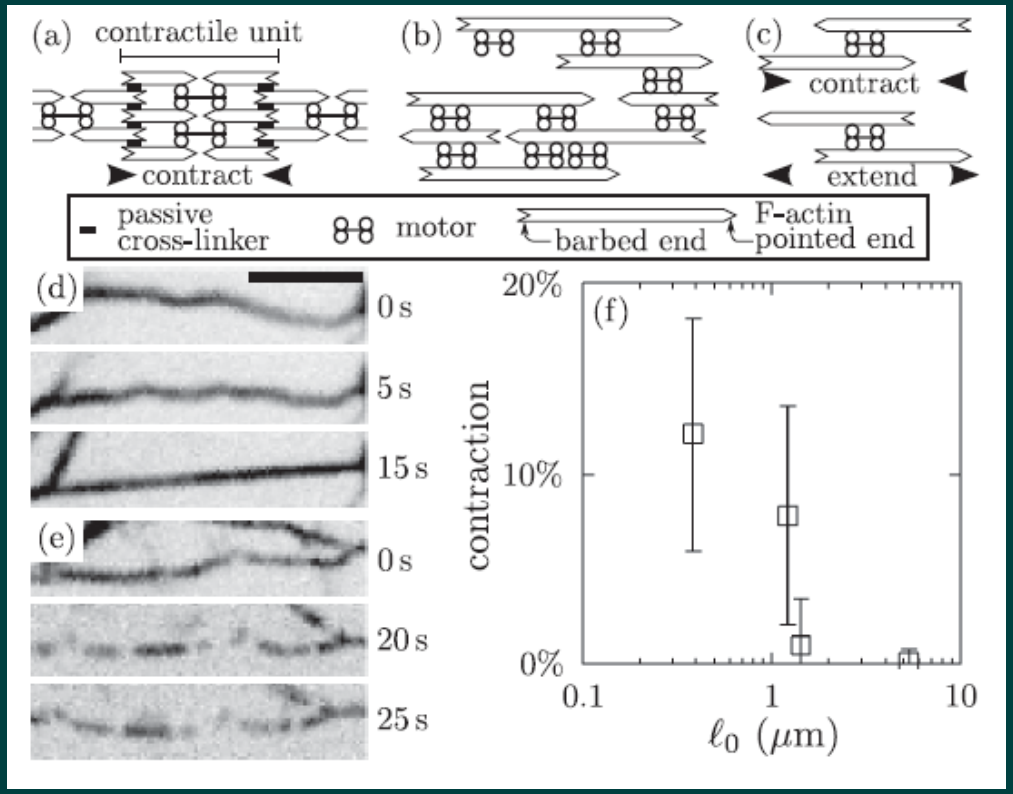
actin/myosin contraction
(analogous to muscle)

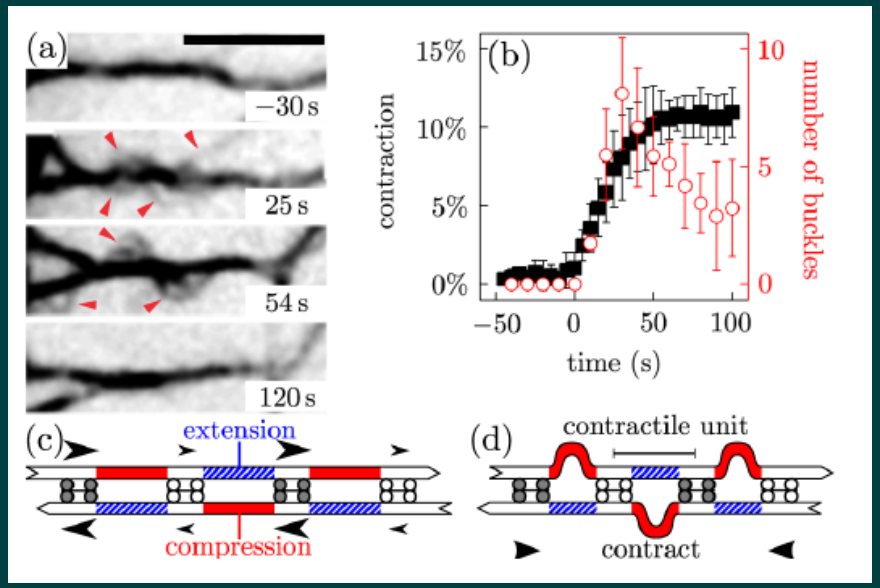
actin assembly

PRL 108, 238107 (2012) PHYSICAL REVIEW LETTERS week ending 8 JUNE 2012

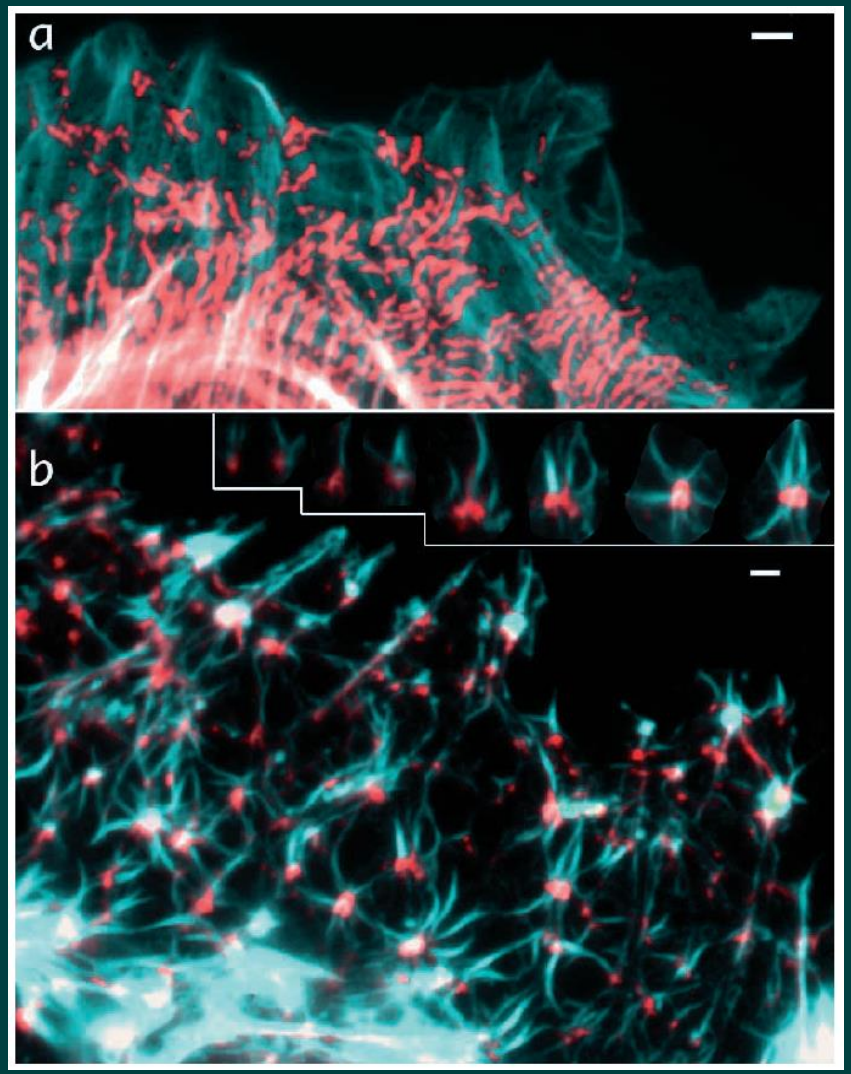
Contractile Units in Disordered Actomyosin Bundles Arise from F-Actin Buckling

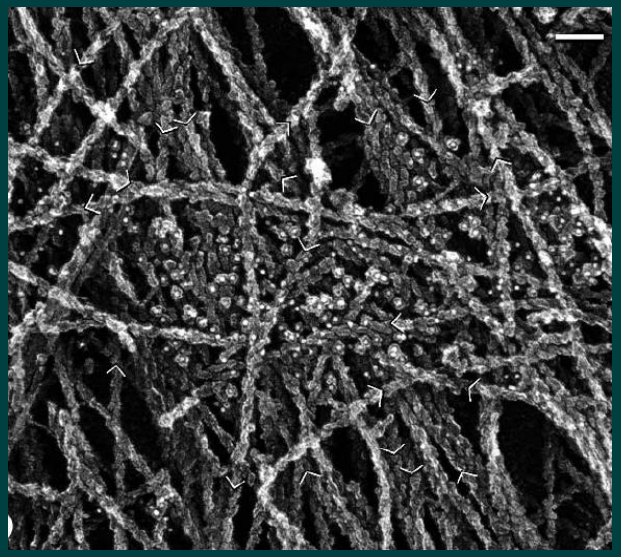
Martin Lenz,[1] Todd Thoresen,[2] Margaret L. Gardel,[1,2,3] and Aaron R. Dinner[1,2,4,*]



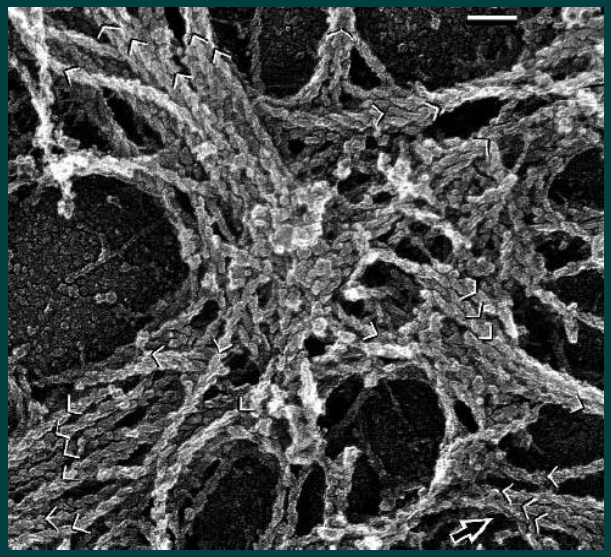


actin-myosin organization in cells





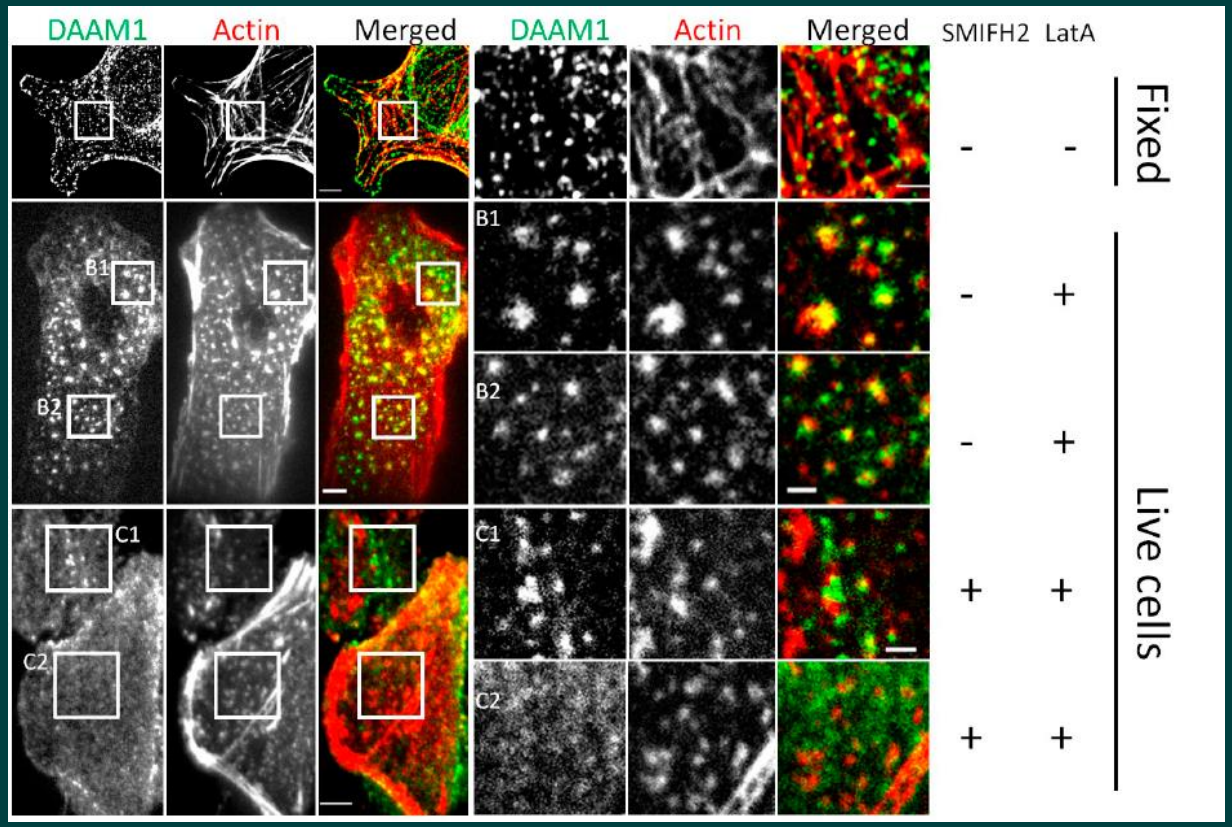
control

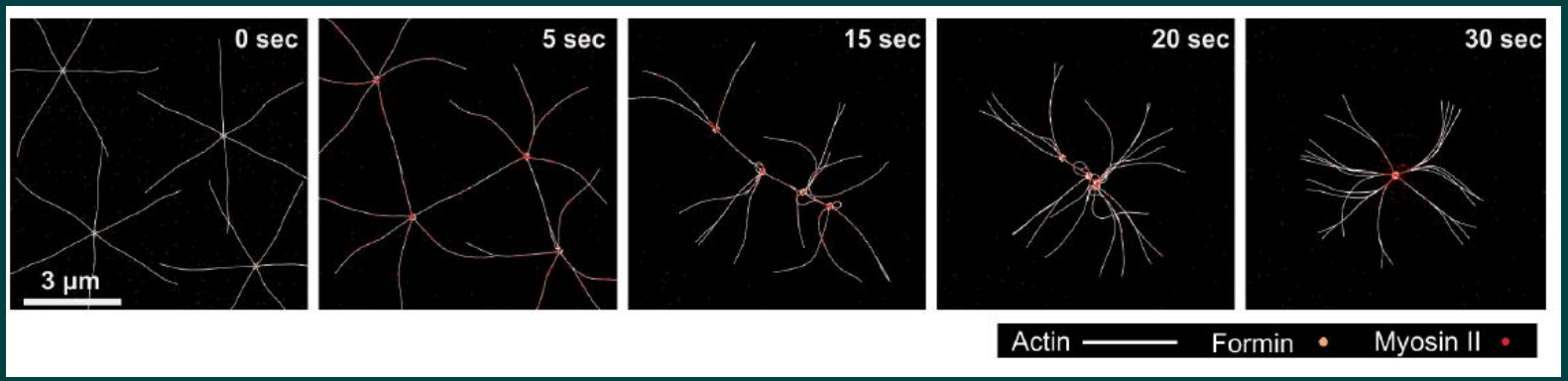


cytochalasin D – formation of asters with pointed ends out

Verkhovsky et al., 1997

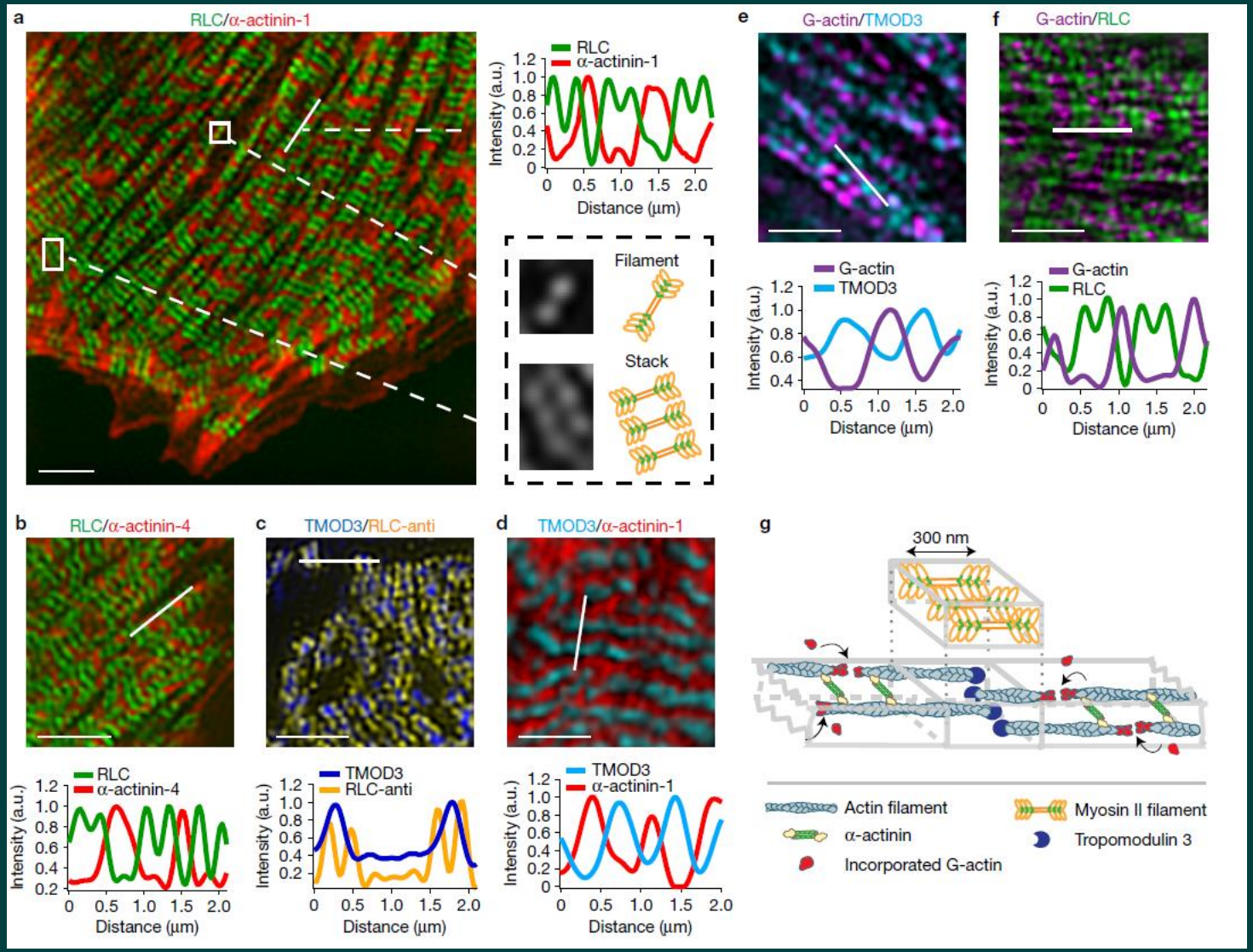
Formin nodes are present before aster formation





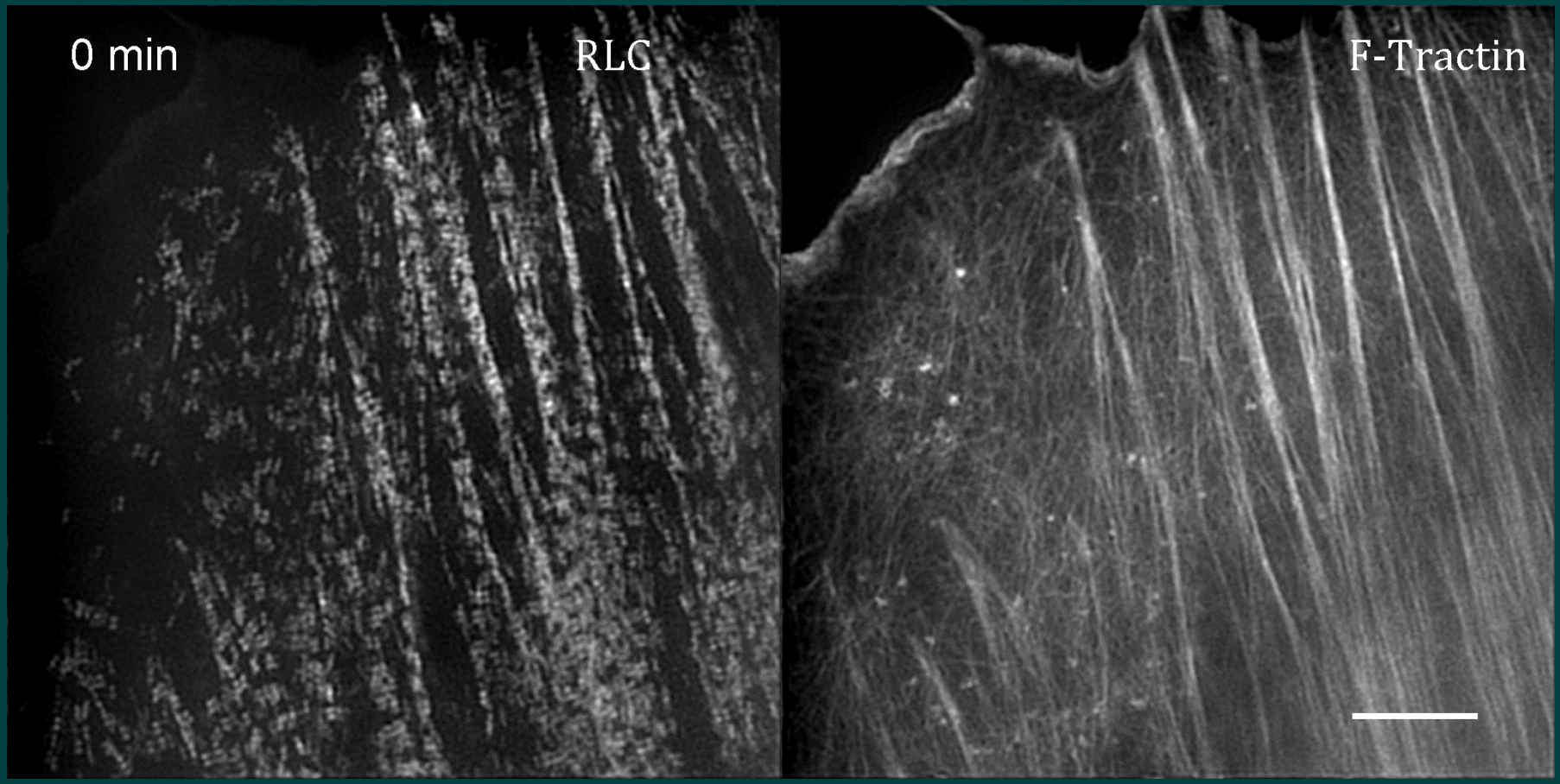
Luo et al., 2016

Super-resolution microscopy of actin-myosin organization: sarcomeric-like arrangement



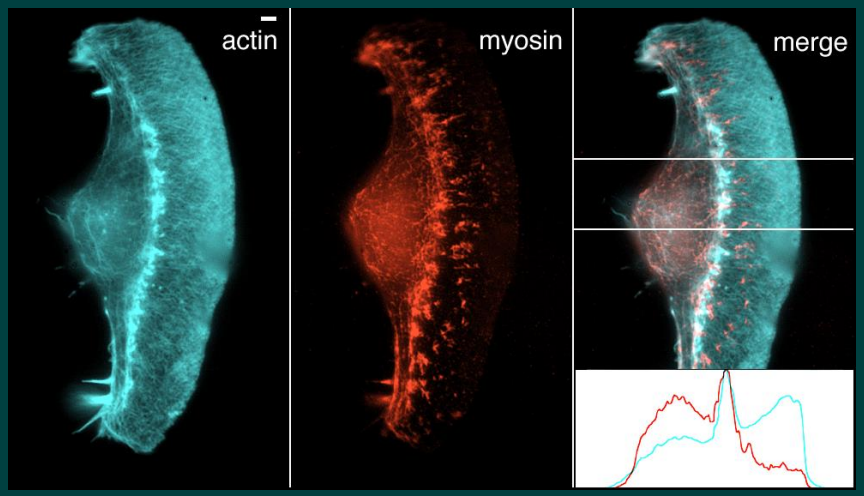
Hu et al., 2017

self-organization of actin-myosin stacks



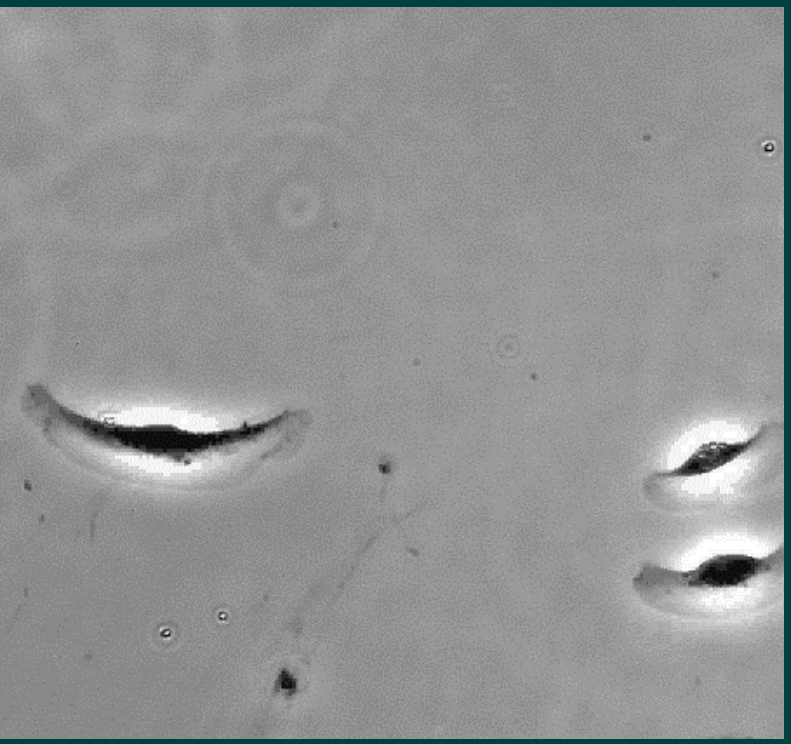
Hu et al., 2017

how myosin II works in cell motion: model of fish keratocytes



Svitkina et al., J. Cell Biol., 1997

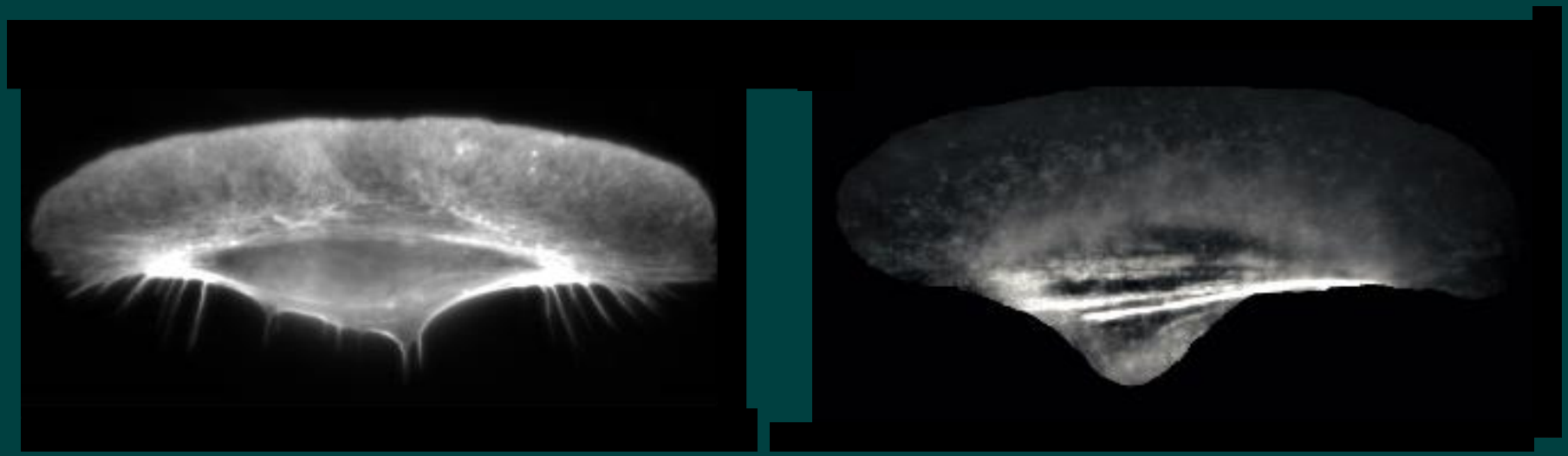
effect of myosin II inhibitor blebbisatin



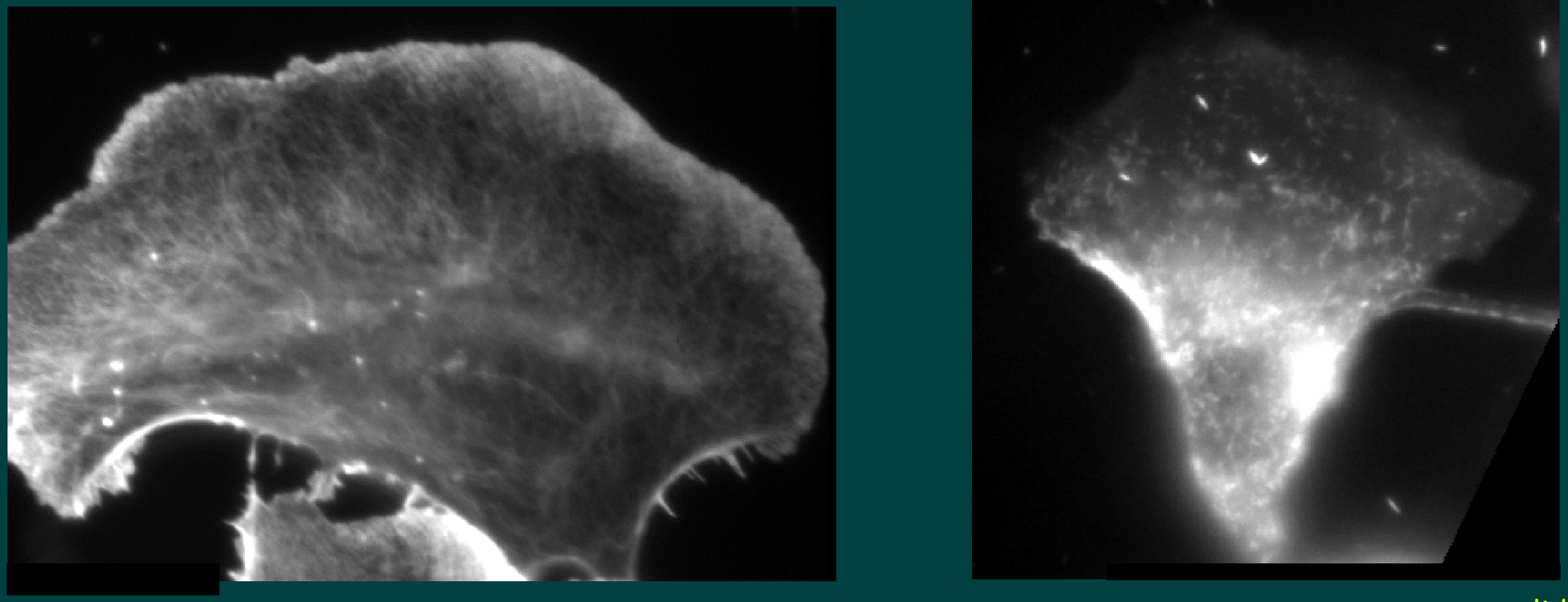
Cells loose coherence and split into fragments

Actin

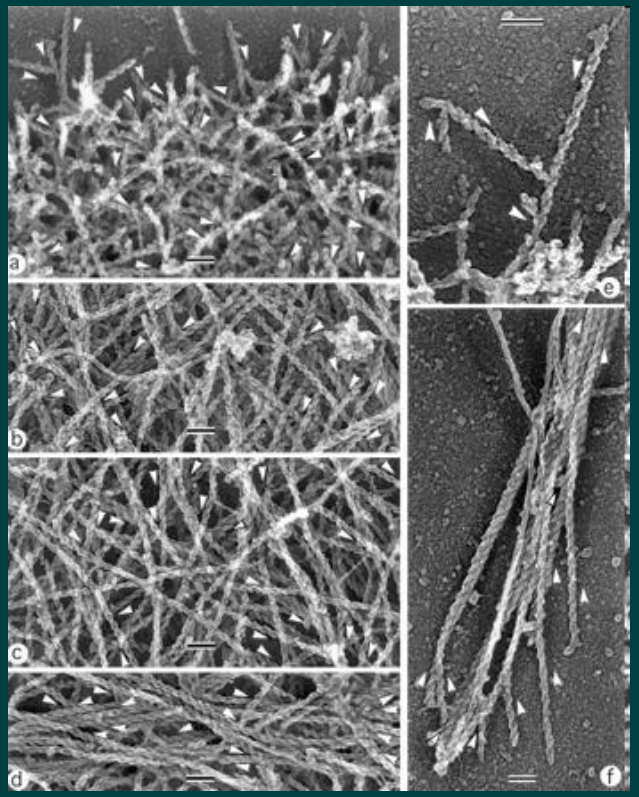
Myosin II

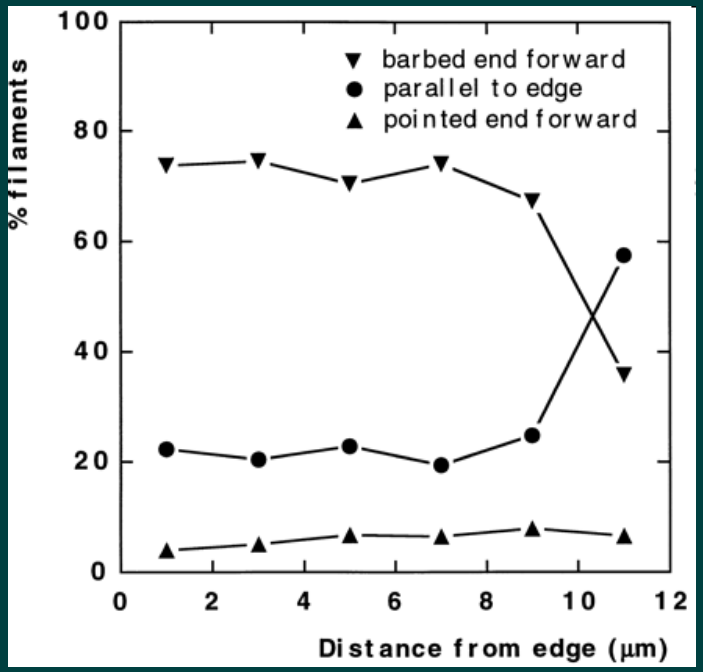


blebbistatin



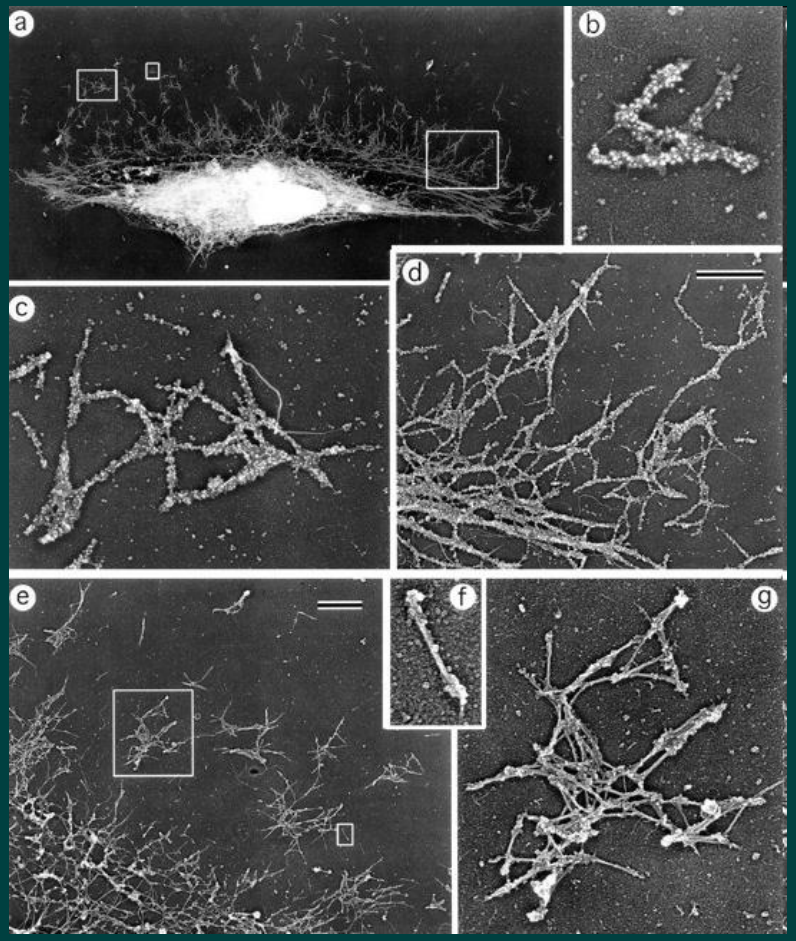
Orientation of actin filaments with respect to the cell and myosin II assemblies: from the front to the back of migrating keratocyte, actin orientation changes from barbed end forward to parallel to the edge

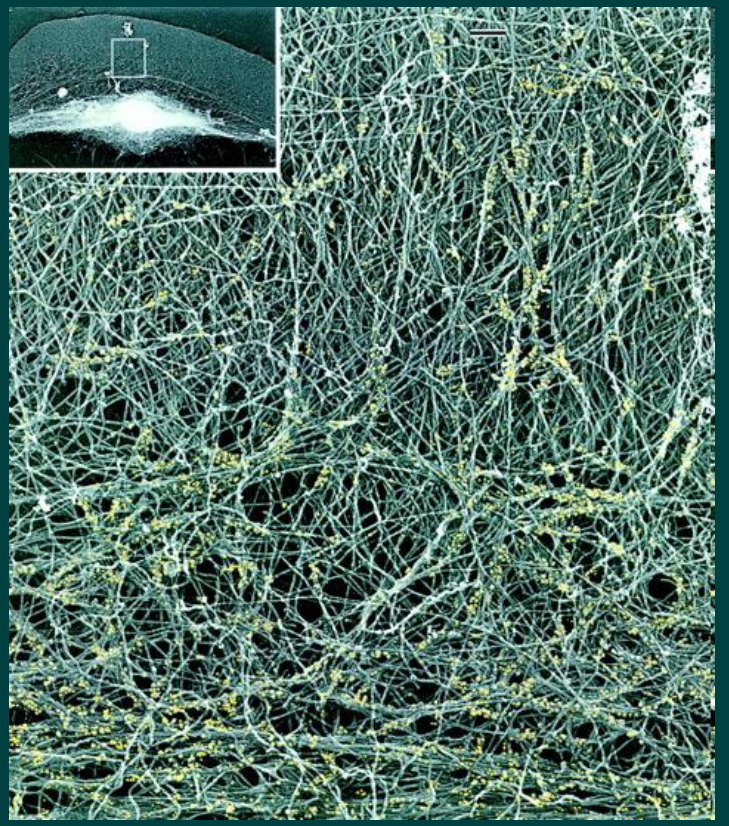




Svitkina et al., JCB, 1997

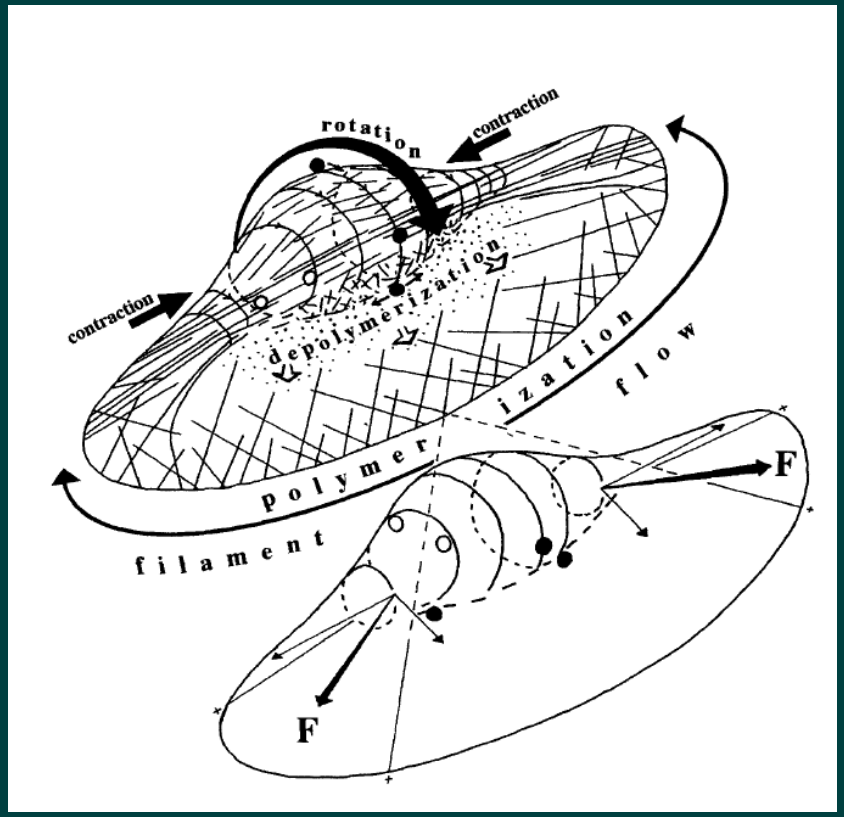
Myosin filament clusters are at the dense intersections of actin network



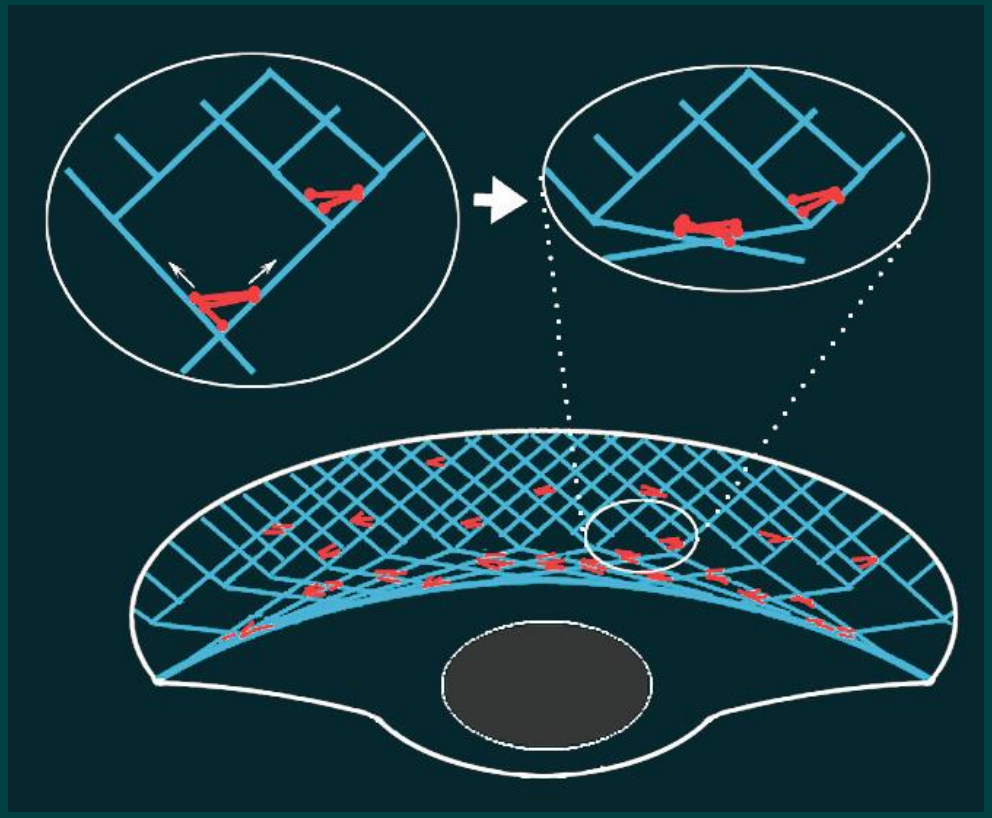


Svitkina et al., JCB, 1997

what brings the cell body forward?



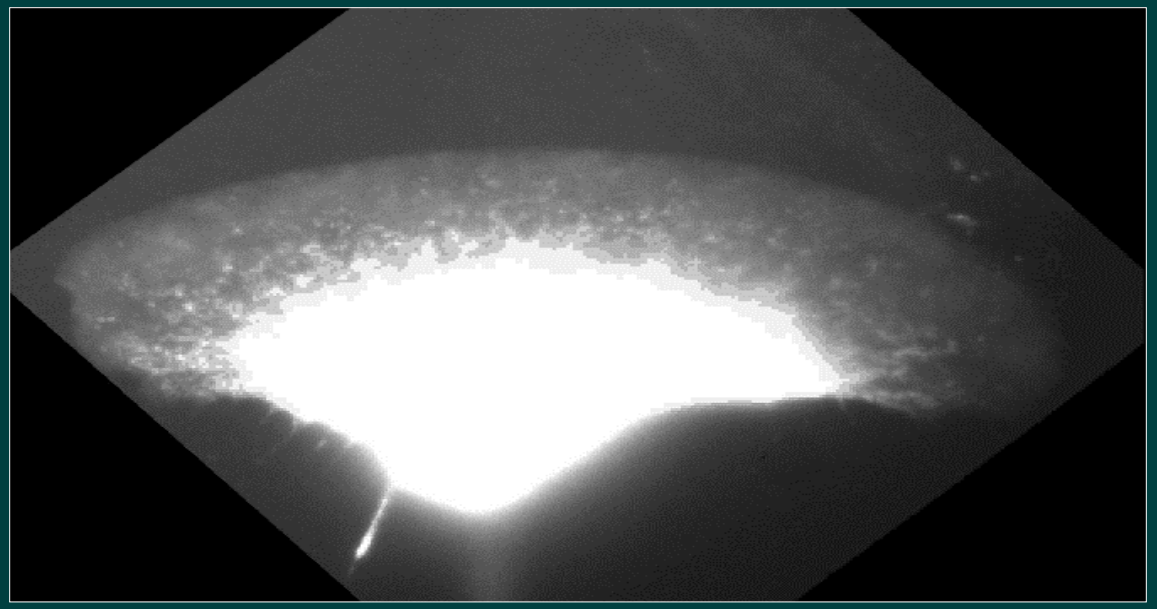
Anderson et al., 96



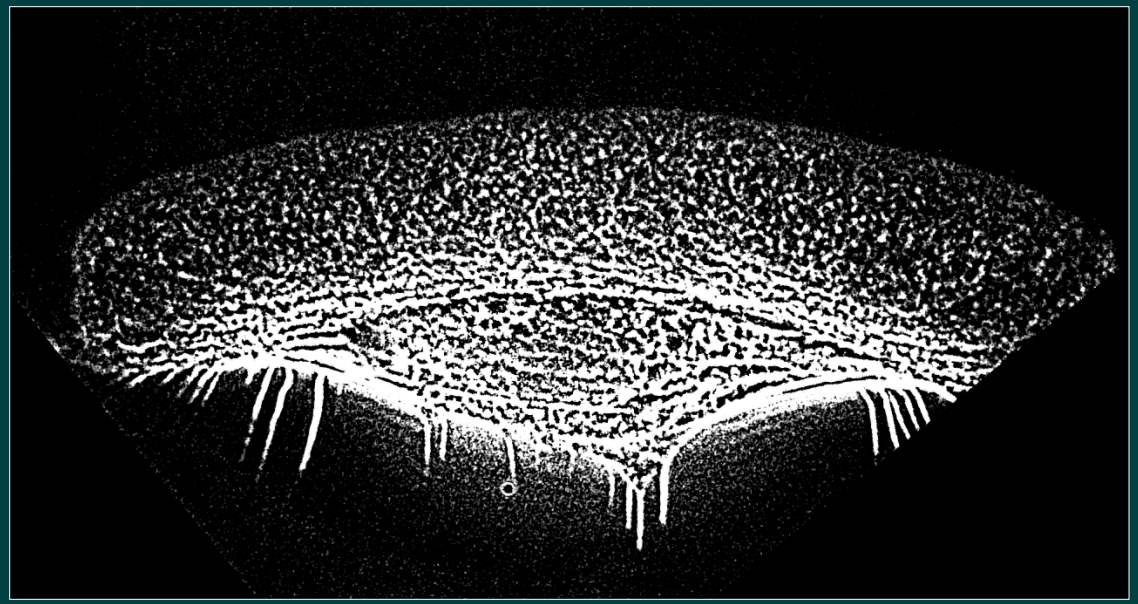
Svitkina et al., 97

actin and myosin II motion tracking

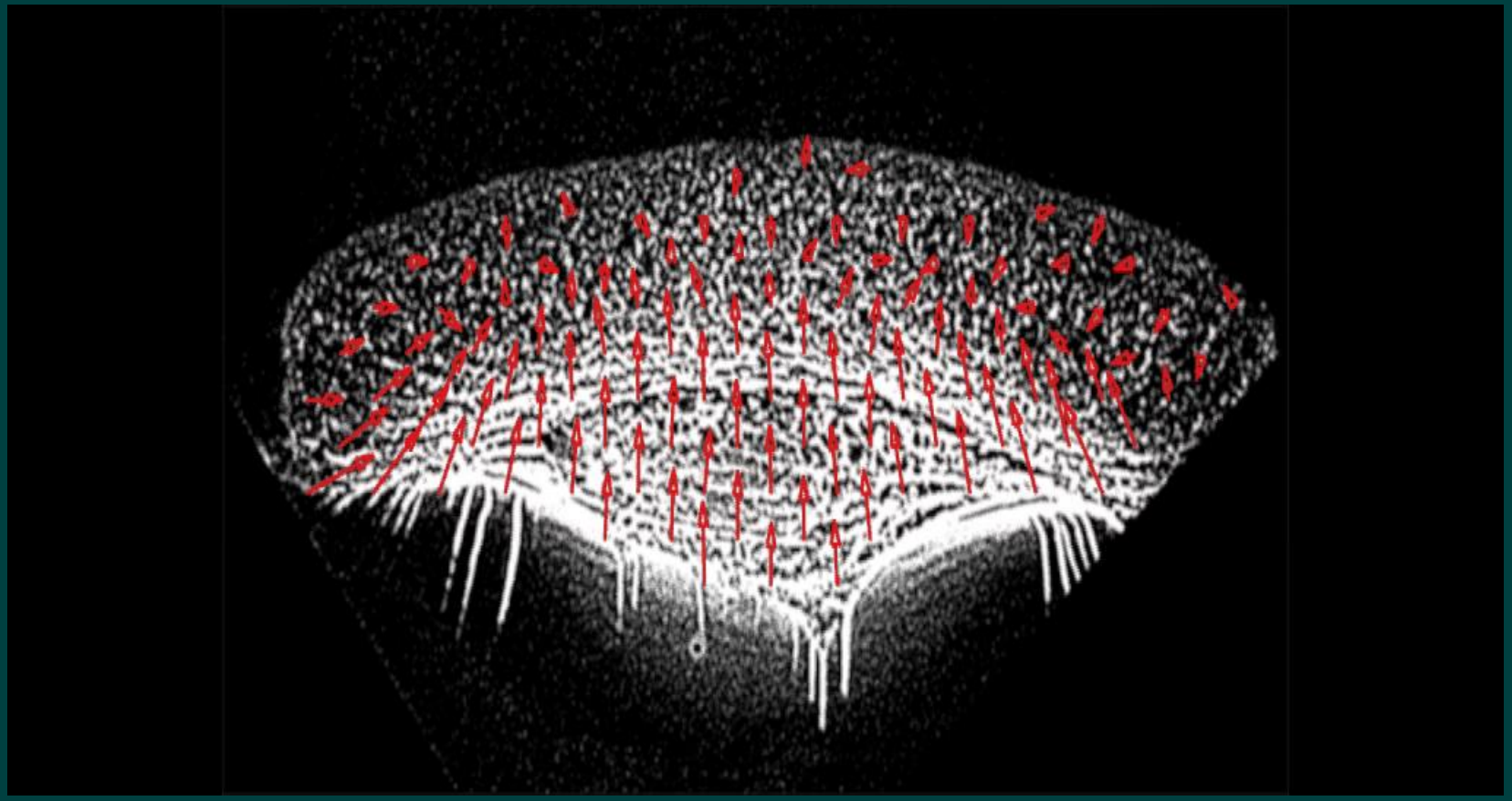
phalloidin injected, raw image



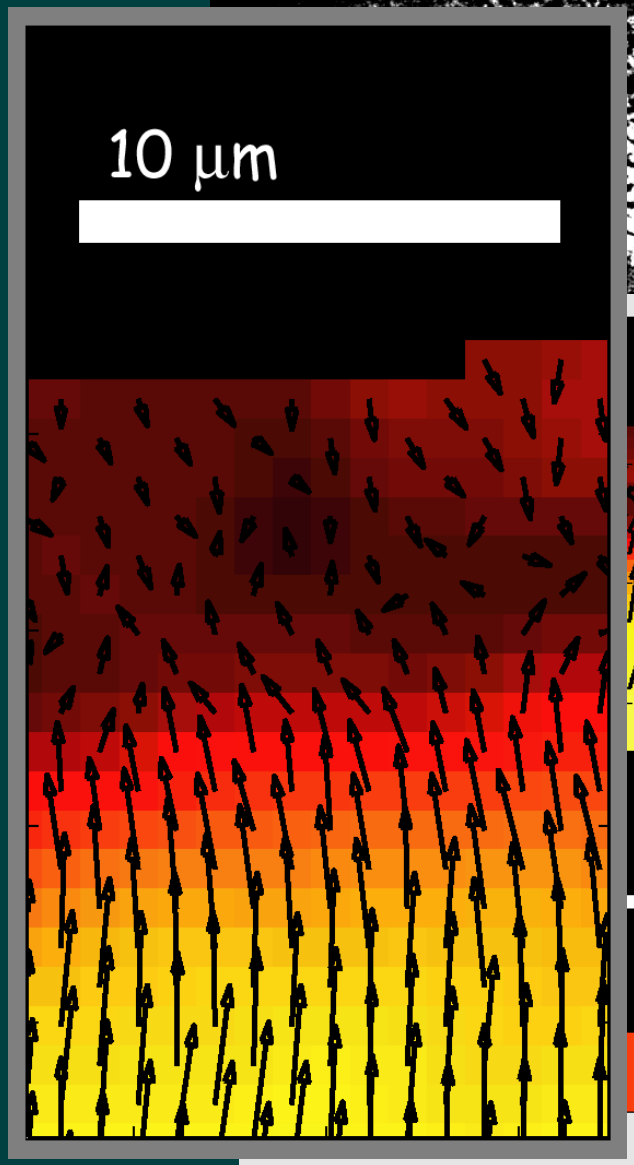
processed (flatten background)

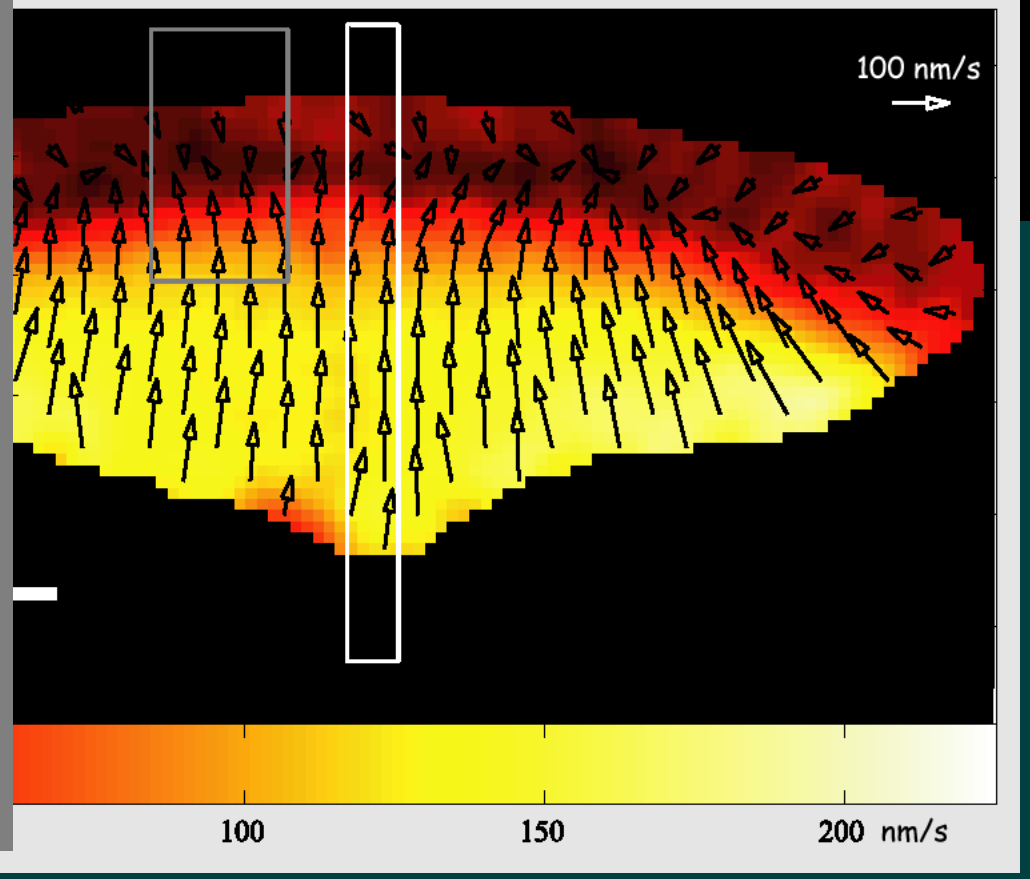


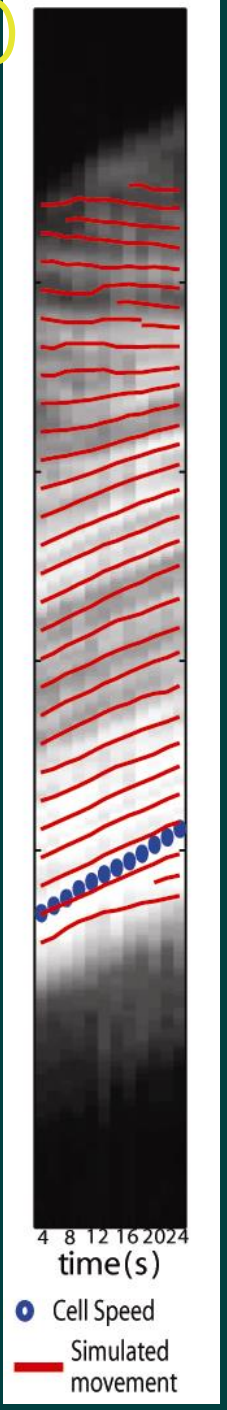
Tracking actin in the entire cell (texture)



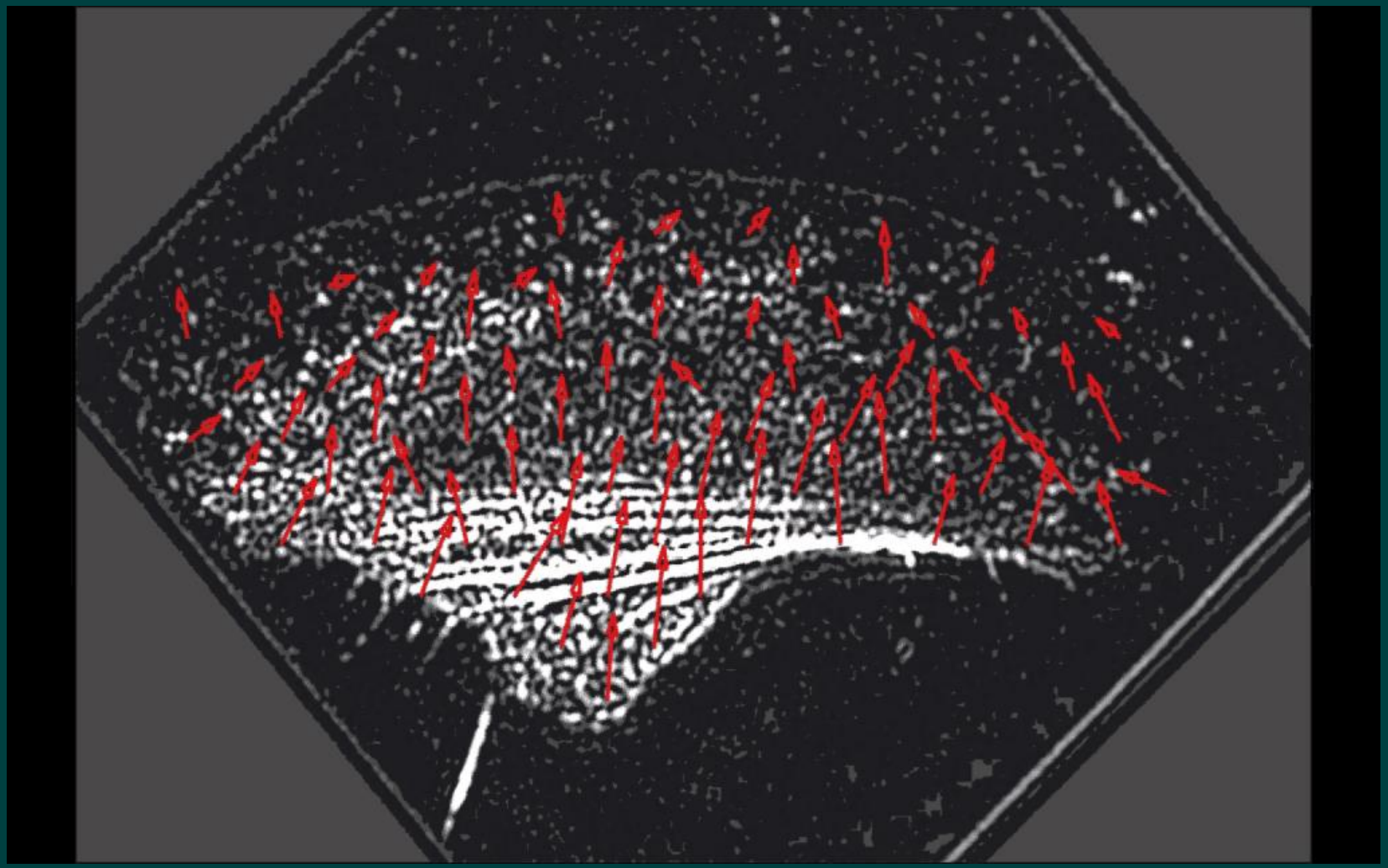
velocity map of the entire cell (texture tracking)





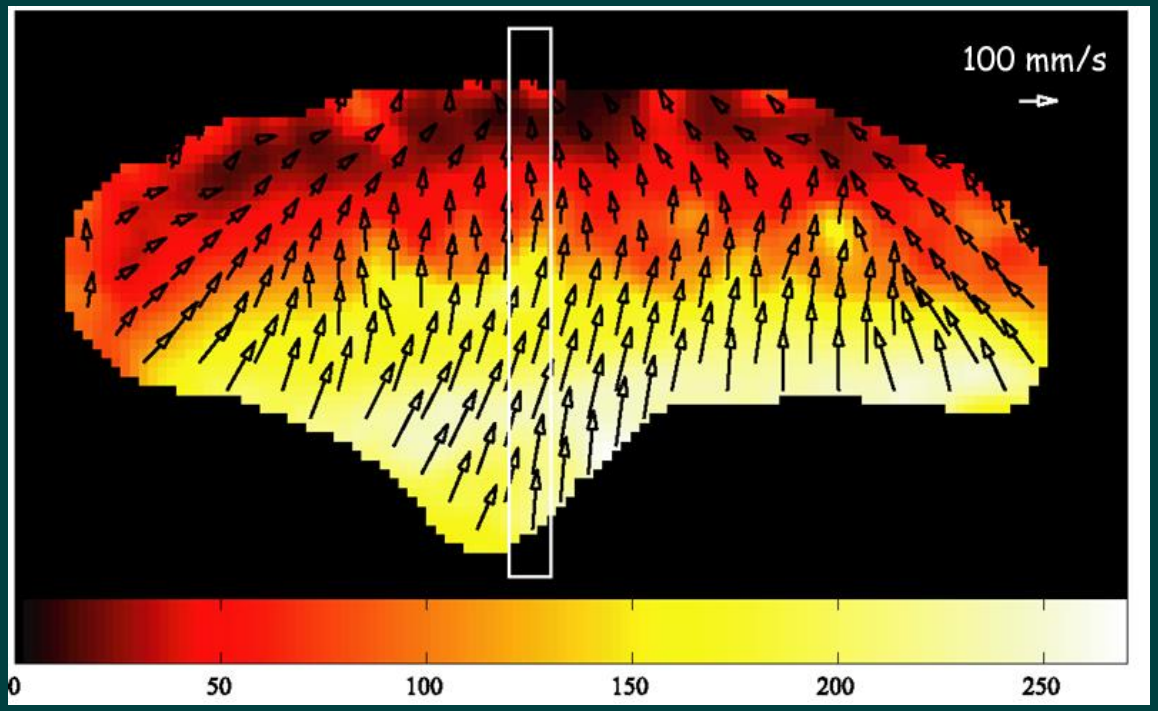


Tracking myosin II

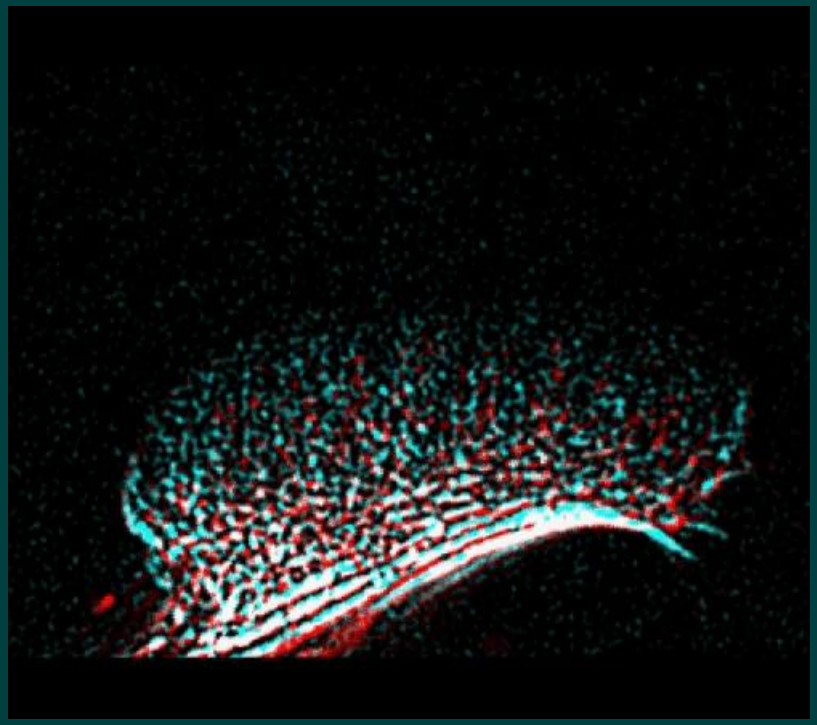


kymograph

myosin II velocity map



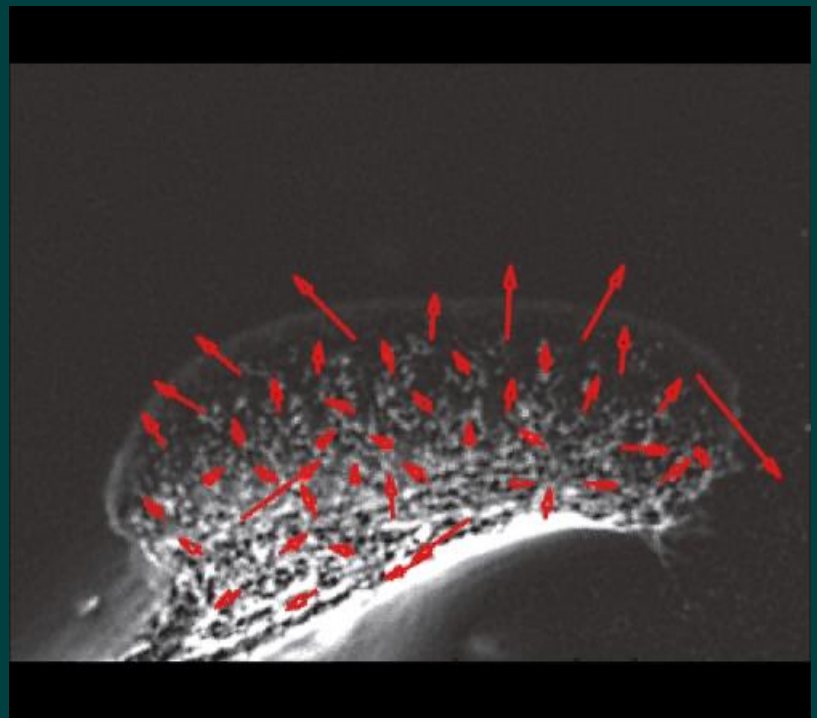
Myosin II moves forward with respect to actin

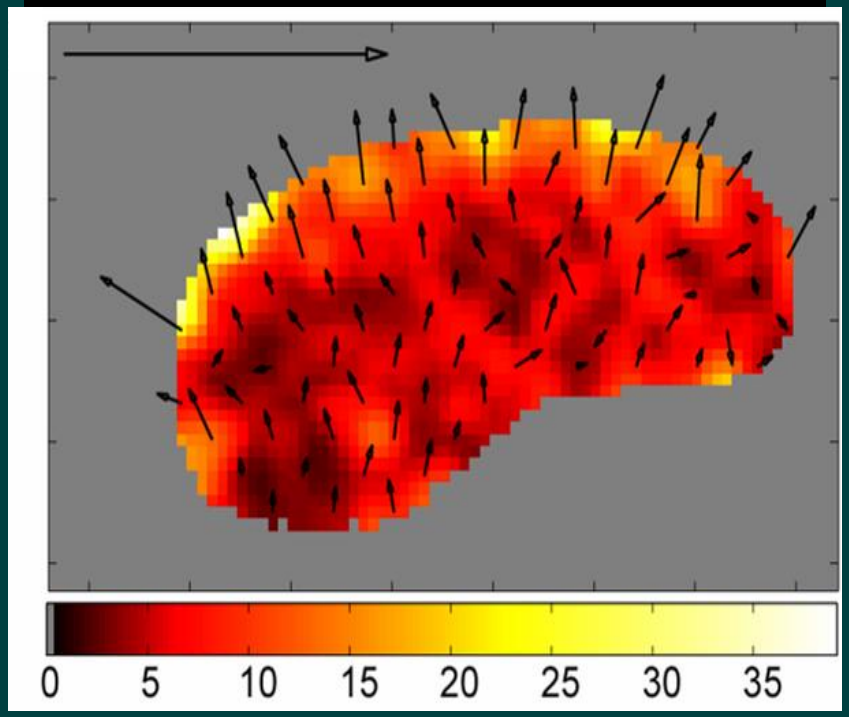


Alexa 488-phalloidin

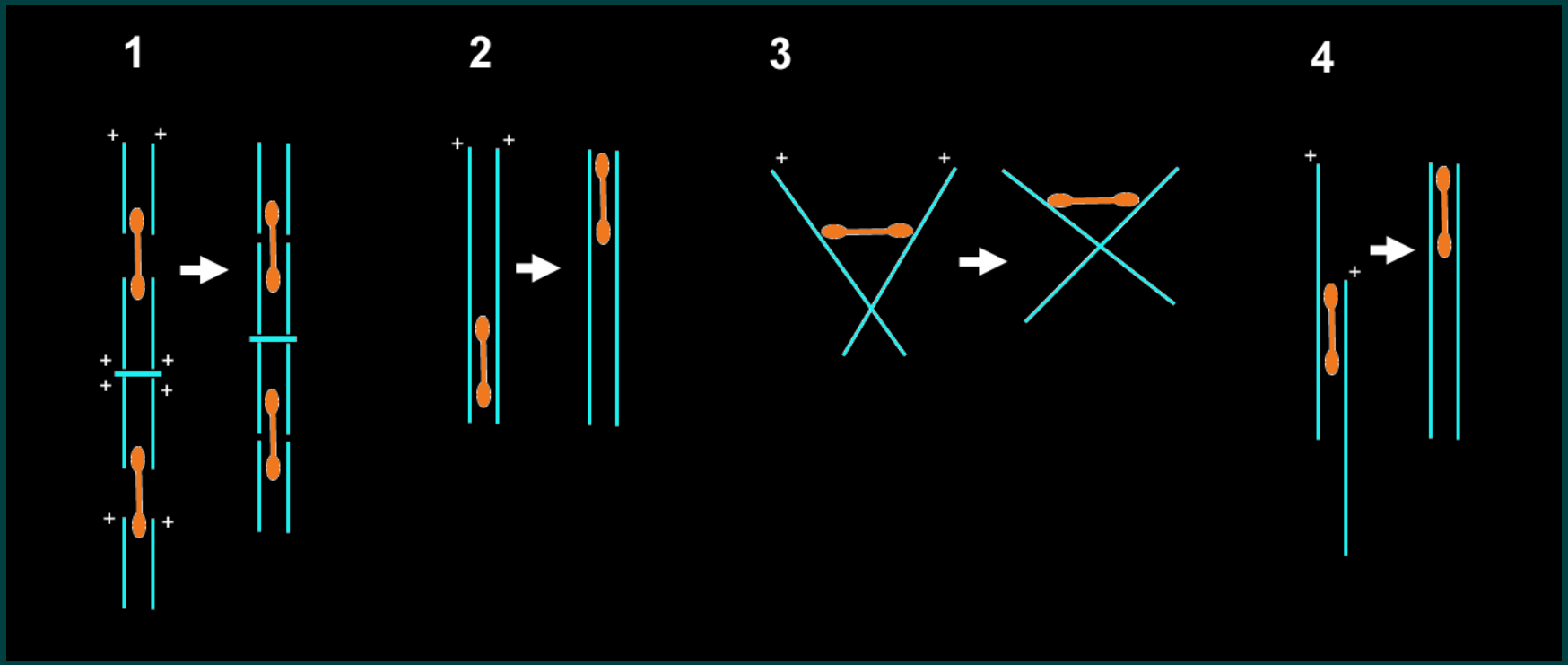
+

rhodamine-myosin II

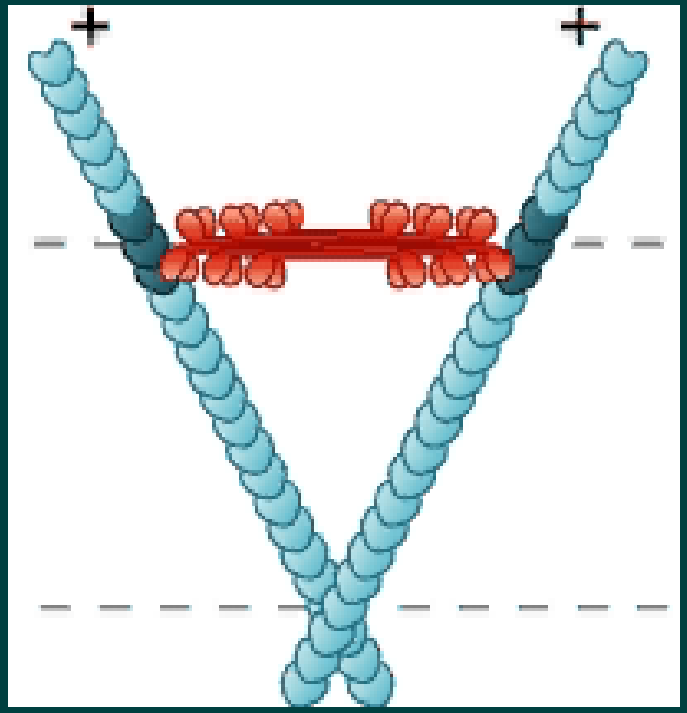




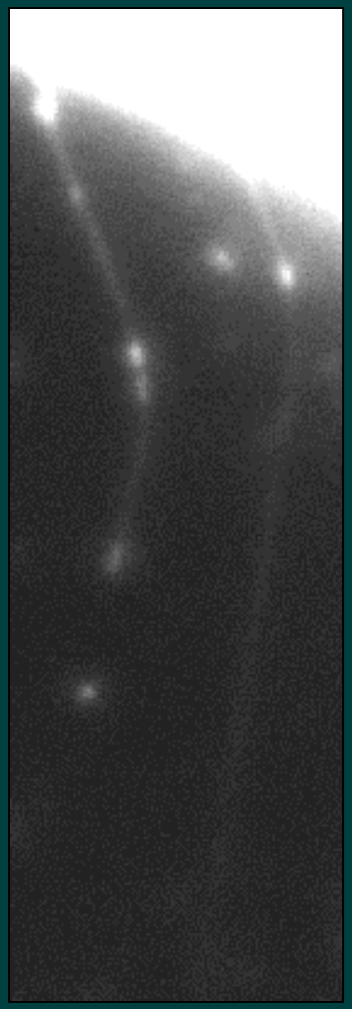
layouts of contractile machinery

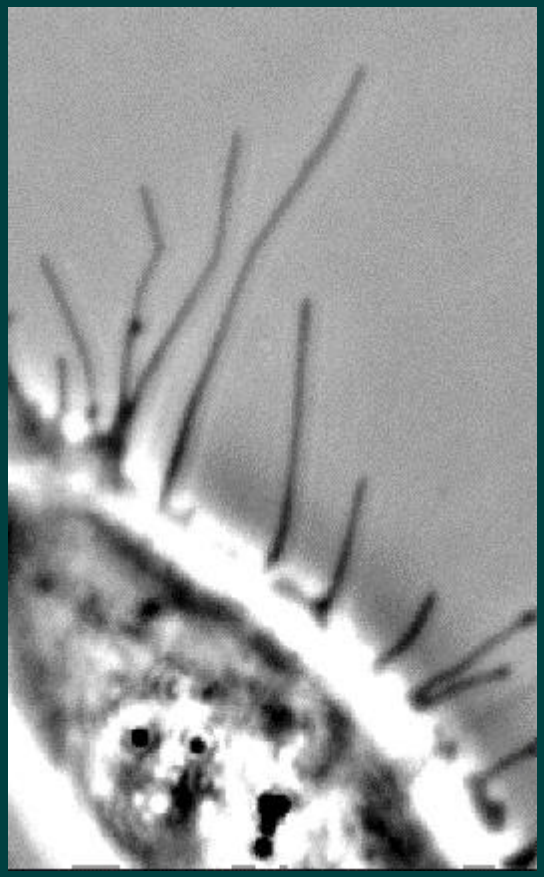


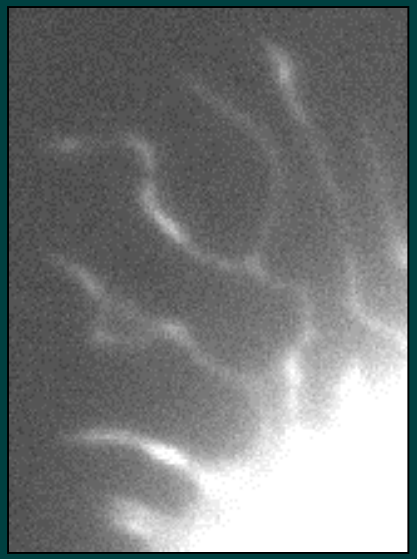
alignment of divergent filaments by myosin motors is consistent with observed motion pattern: both actin and myosin move forward, but myosin is a little faster than actin



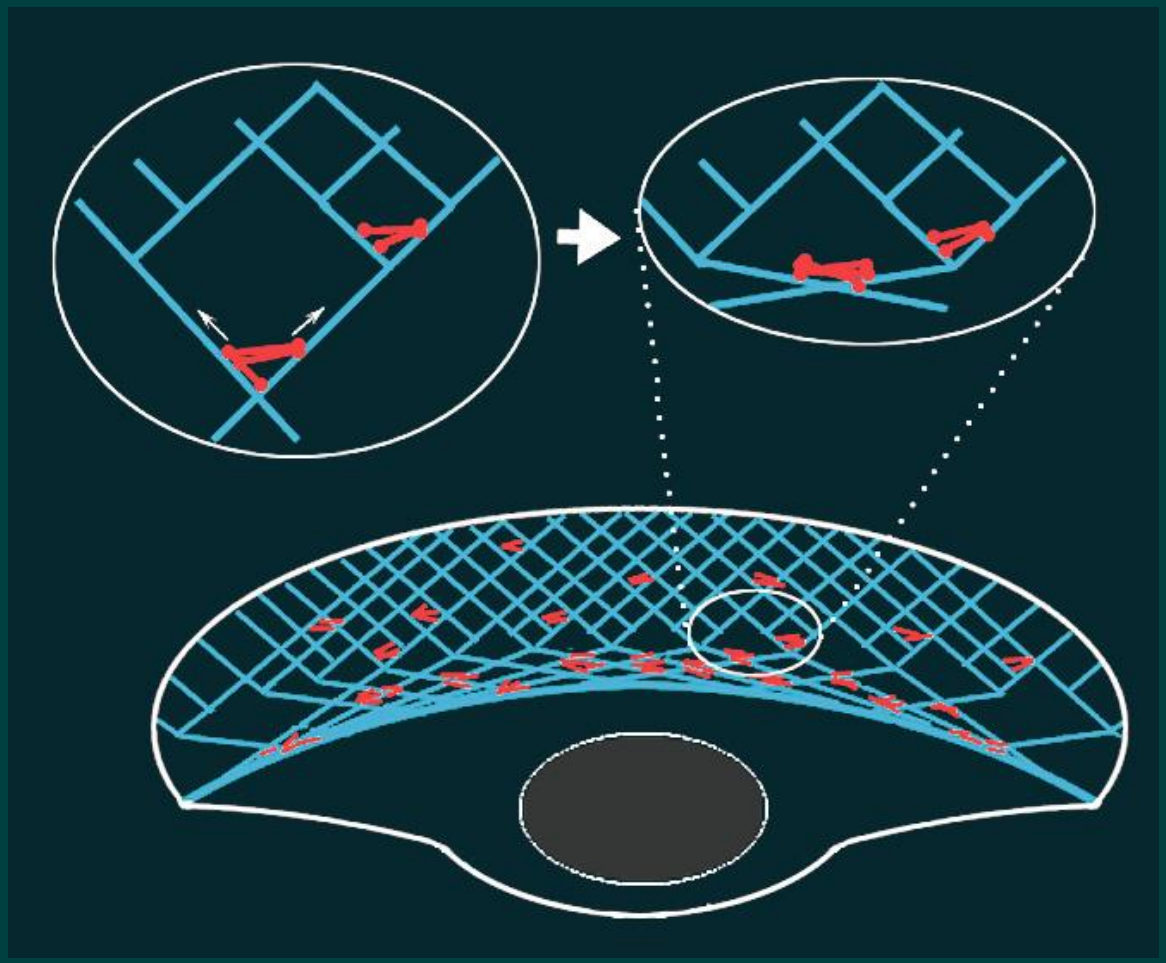
motion along distinct actin tracks: myosin II in retraction fibers

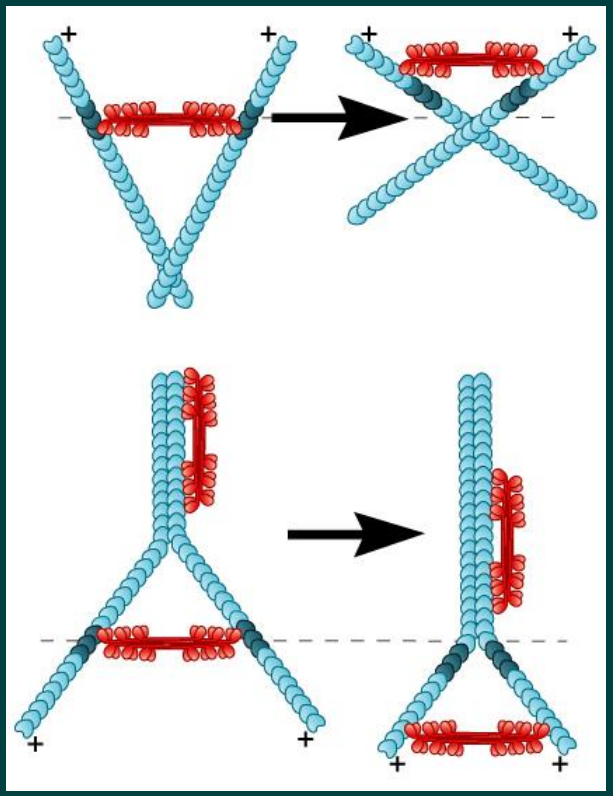






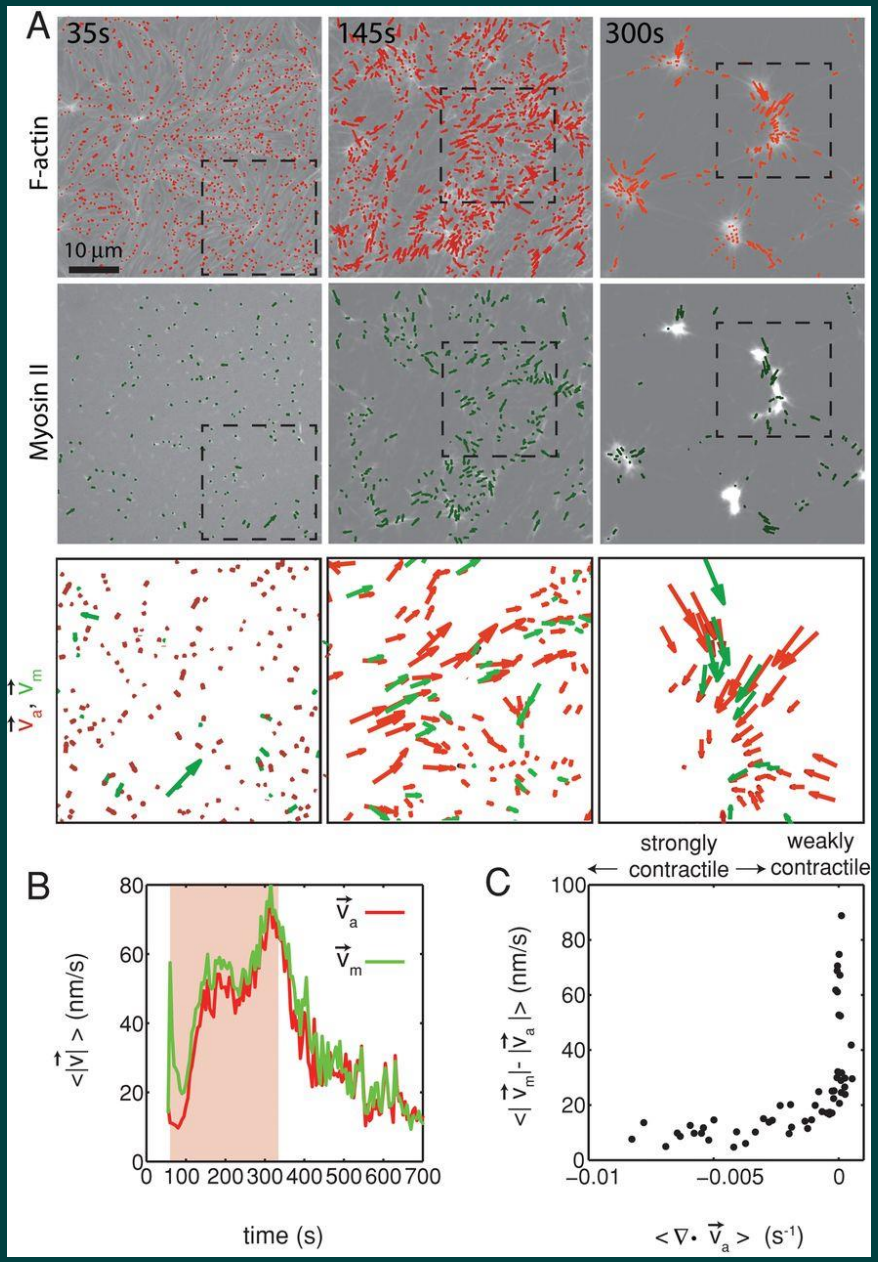
contraction mechanism

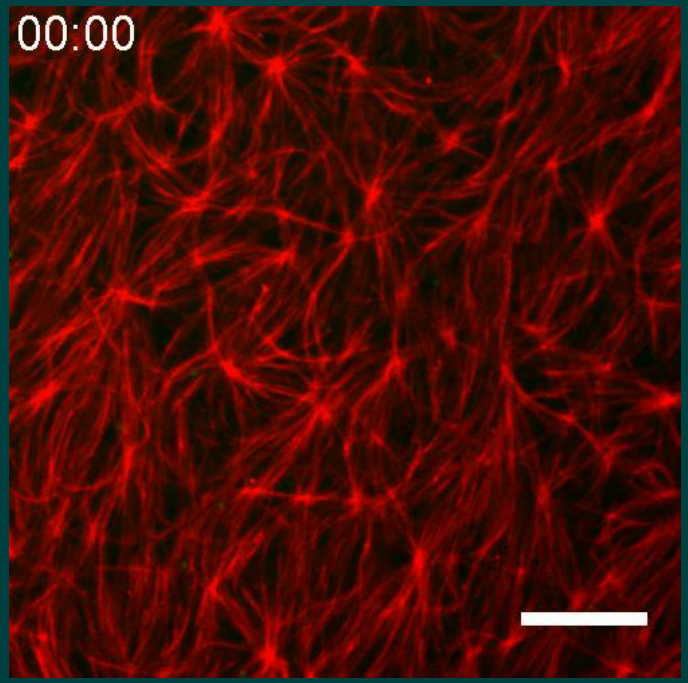




Schaub, Bohnet et al., 2007

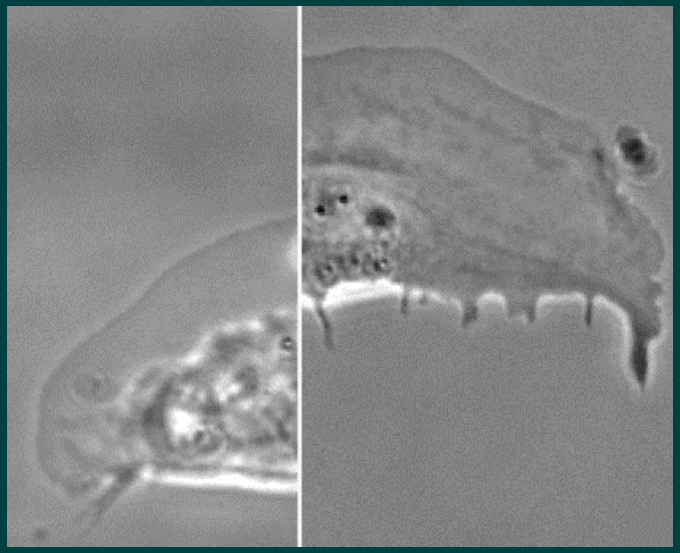
in vitro: relative actin-myosin motion is attenuated during contraction





Murrelle and Gardel, 2014

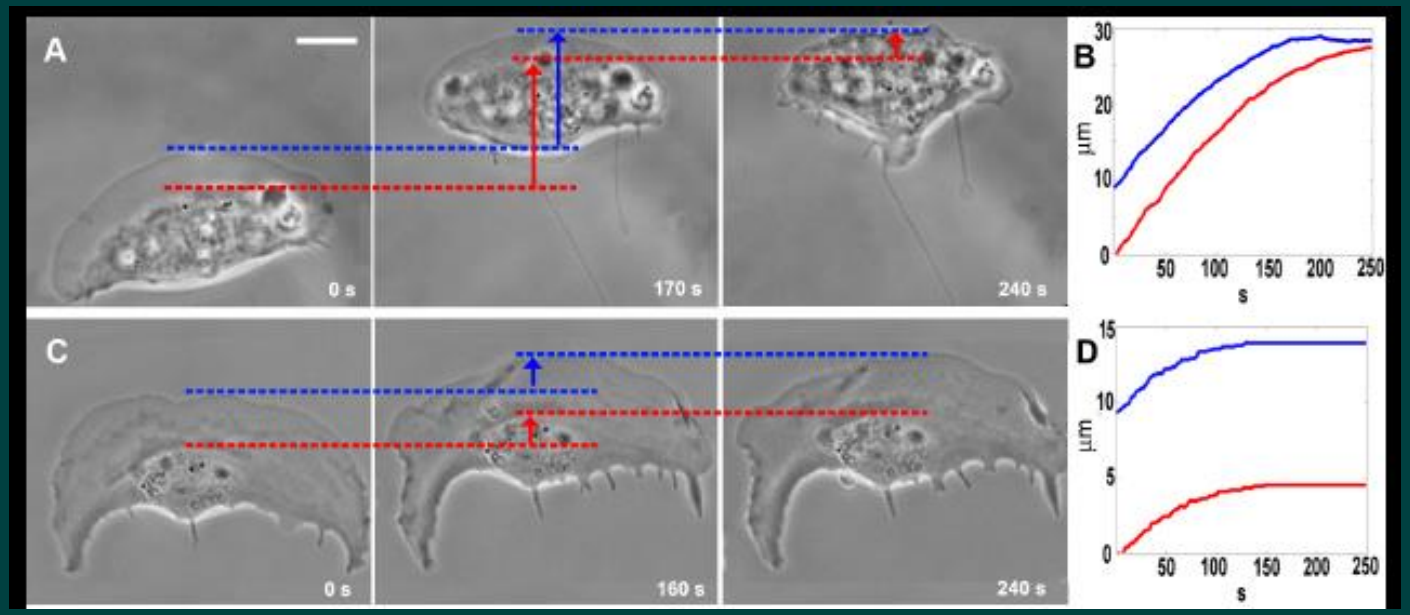
cytochalasin D



blebbistatin
+ cytochalasin D

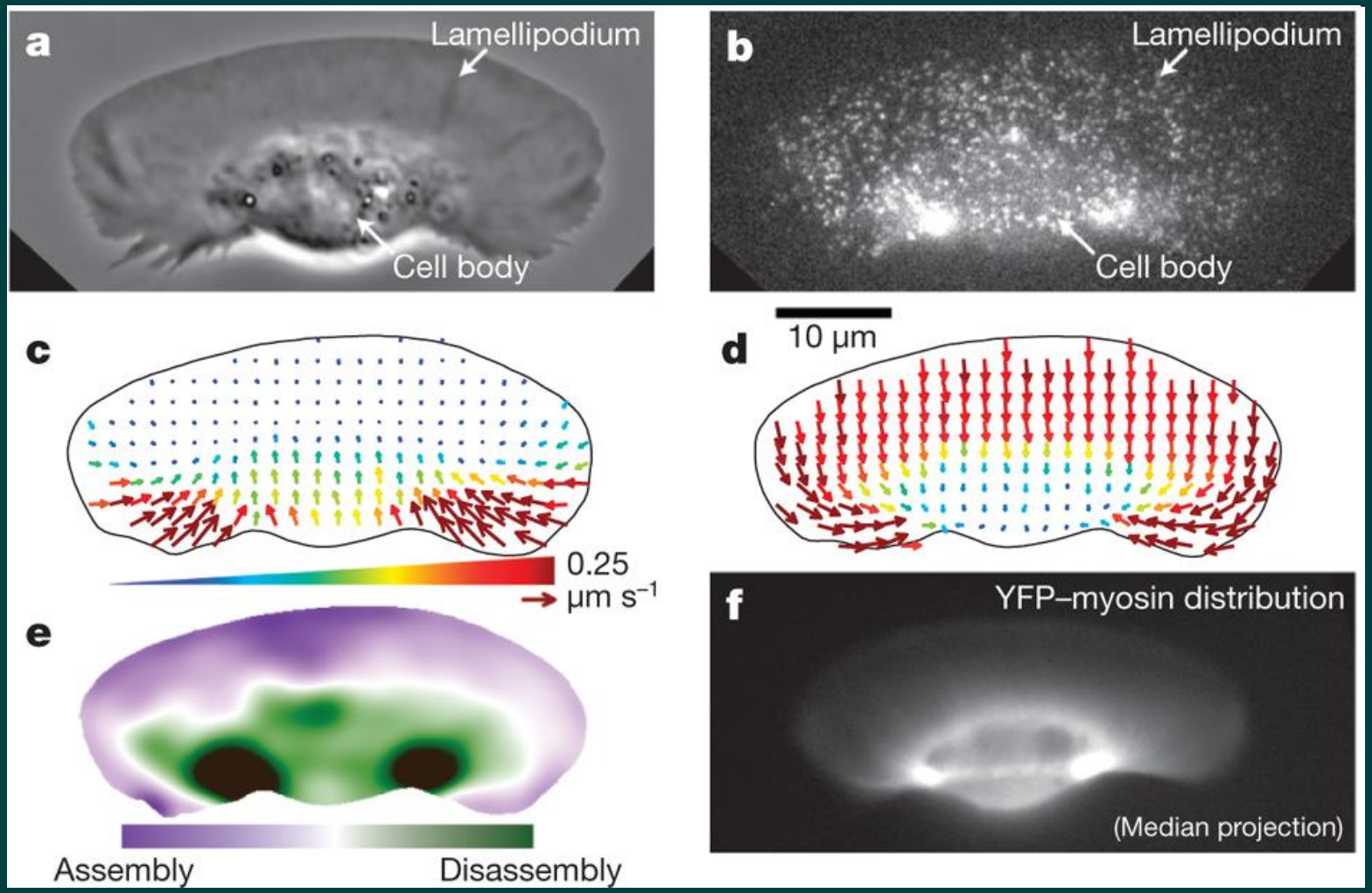
cytochalasin D

blebbistatin
+ cytochalasin D



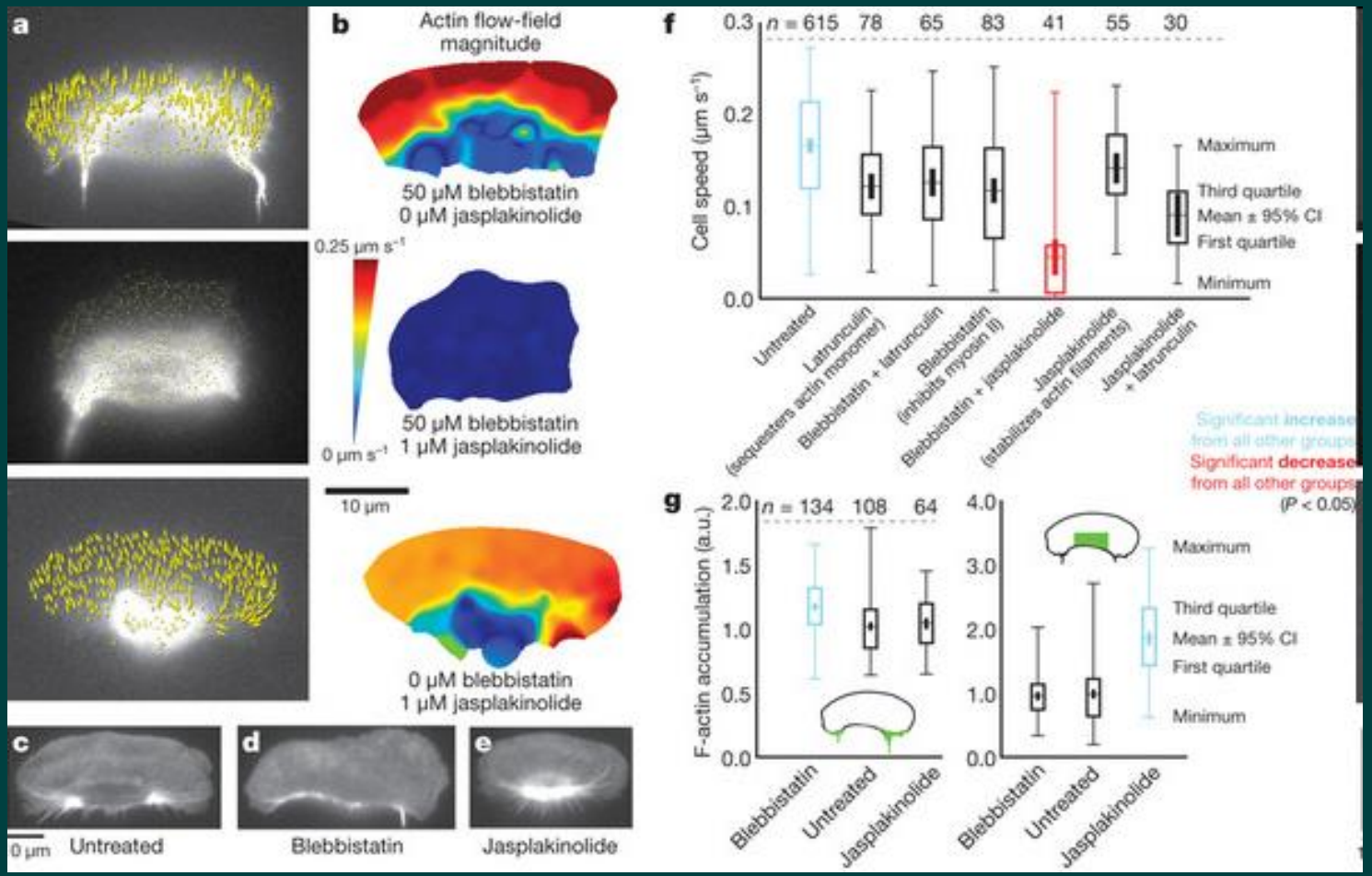
When myosin II does not work, cell body translocation becomes fully dependent on front protrusion (Fournier et al., 2010)

Additional myosin II function: contribution to actin disassembly



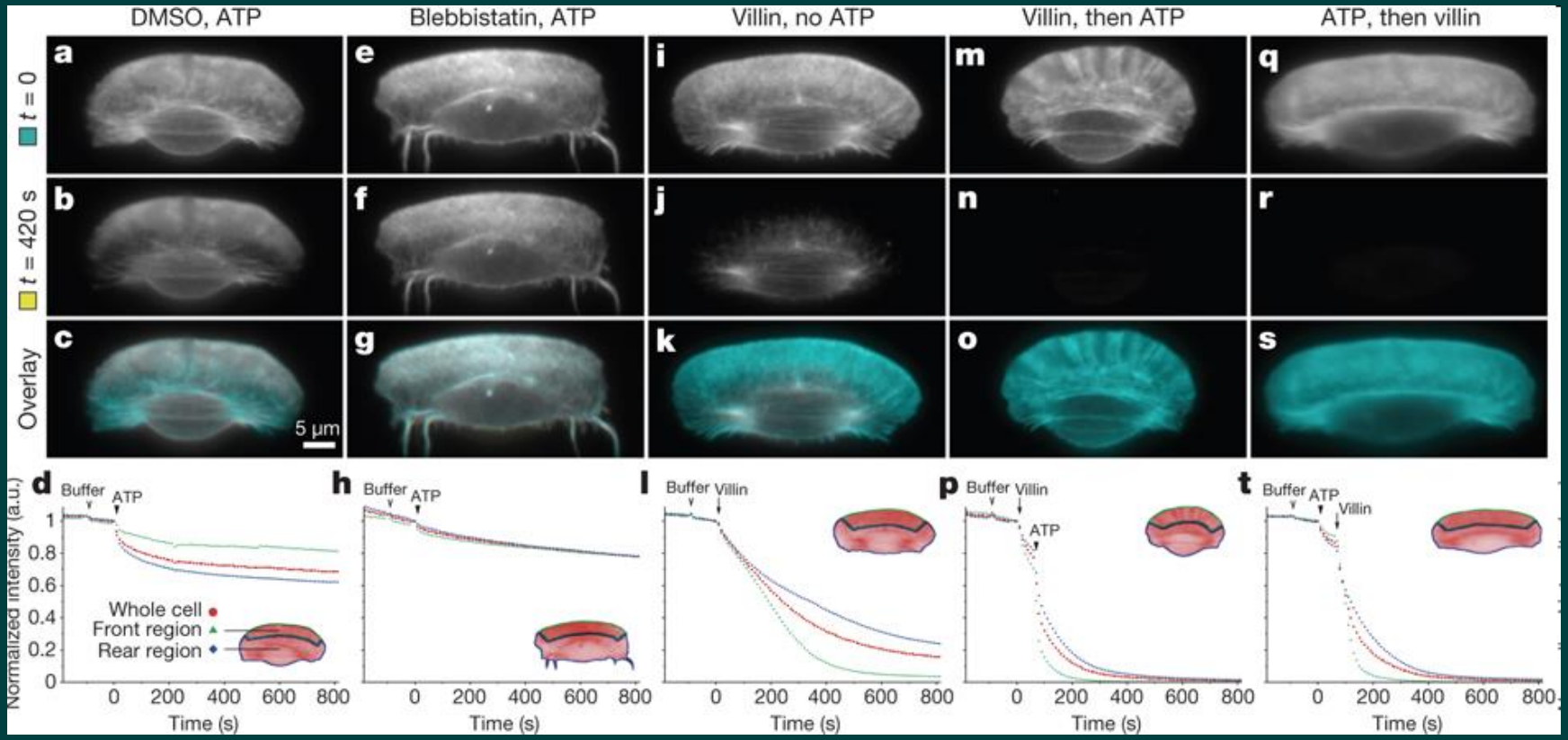
Myosin II coincides with actin disassembly sites

Wilson et al, 2010



Myosin II inhibitor blebbistatin and actin disassembly inhibitor jasplakinolide together inhibit cell motion and actin turnover

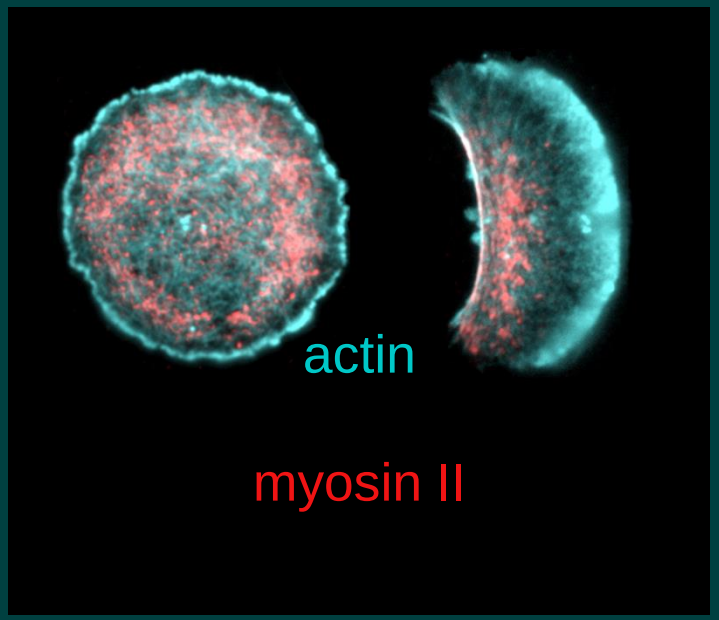
Wilson et al., 2010



actin disassembly in permeabilized cytoskeletons is ATP-dependent and blebbistatin-sensitive

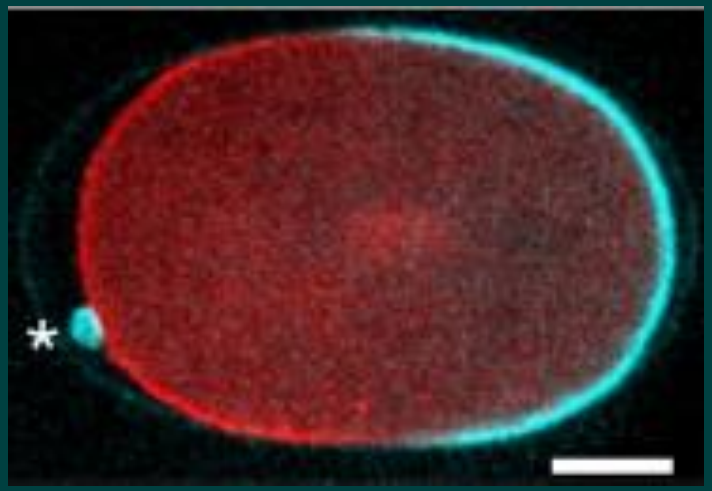
Wilson et al., 2010

Why myosin II is at the back?

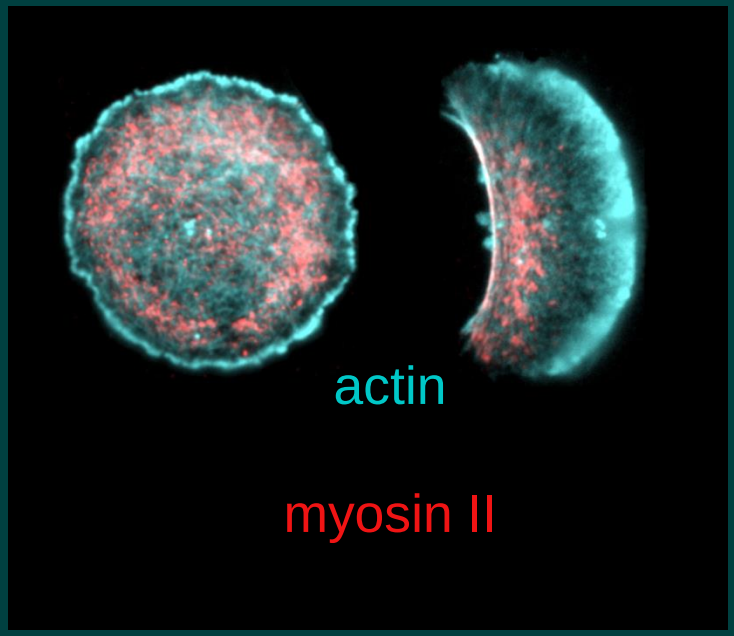


in migrating cell directional cytoskeletal pattern arises during polarization

polarization is a general phenomenon



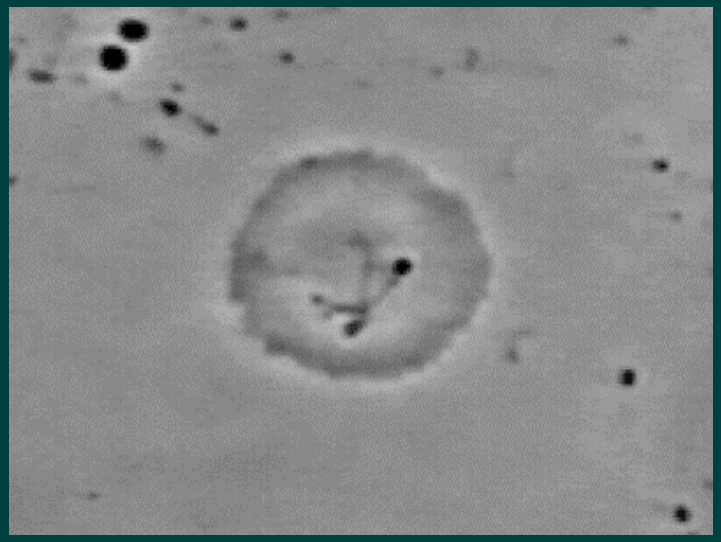
Asymmetric fertilized oocyte
of C. elegans
(PAR proteins)

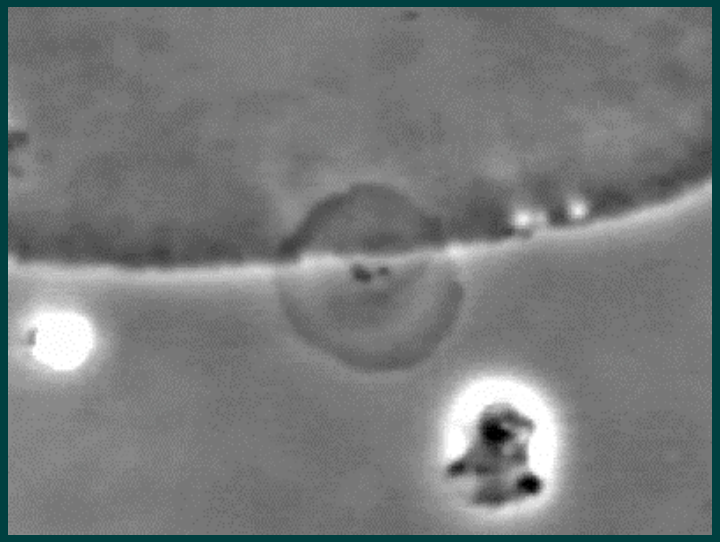


Motile cell fragment

How do millions of molecules spontaneously segregate against diffusion?

Positive feedback between motion and polarity



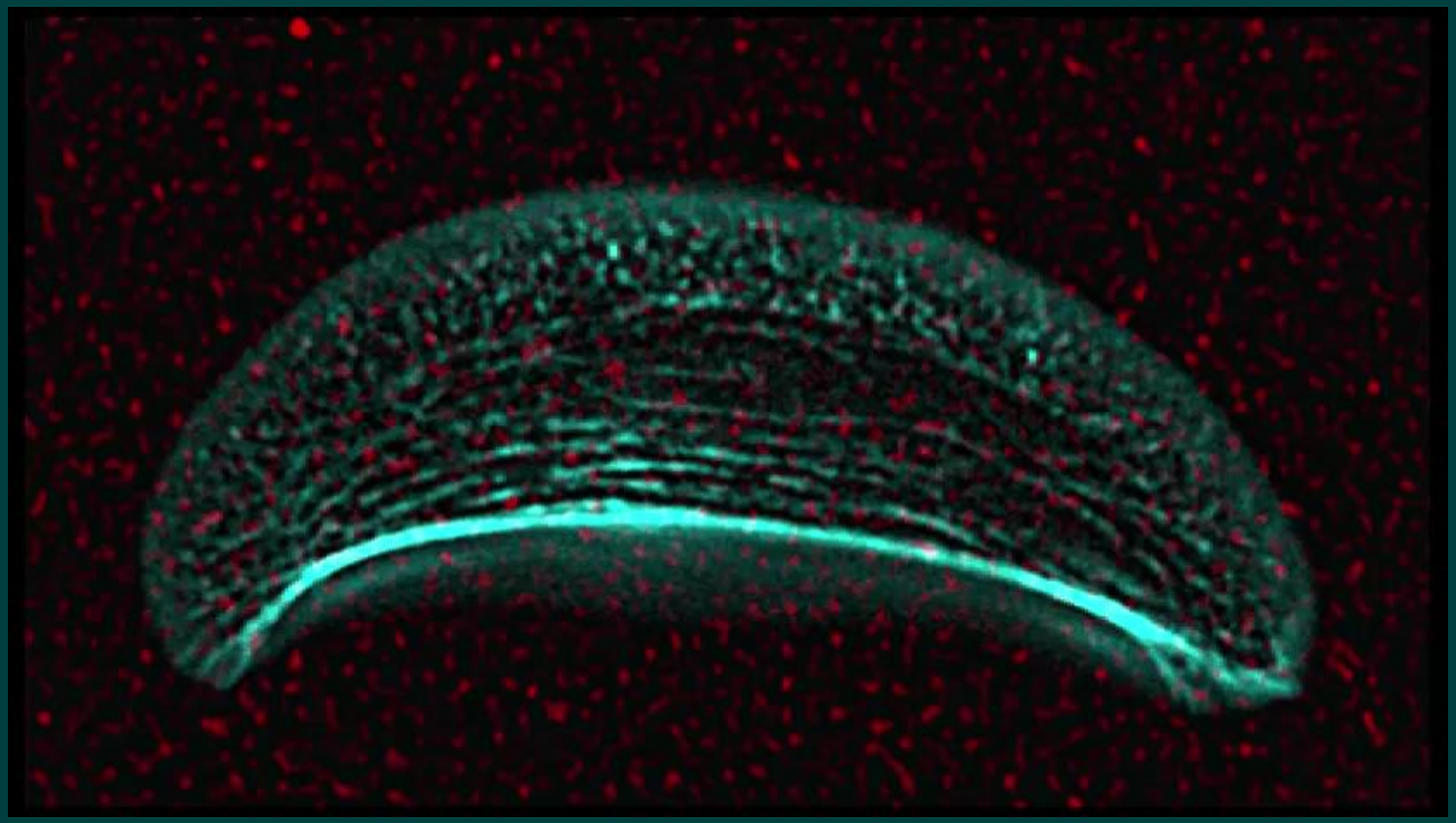


Verkhovsky et al., Curr. Biol., 1999

next time presentation: recent study investigating and proving the relationship between polarity and motion (Maiuri et al., 2015)

How are the forces transmitted to the substrate?

substrate stress could be estimated from deformation



Gelatin substrate (Doyle and Lee)

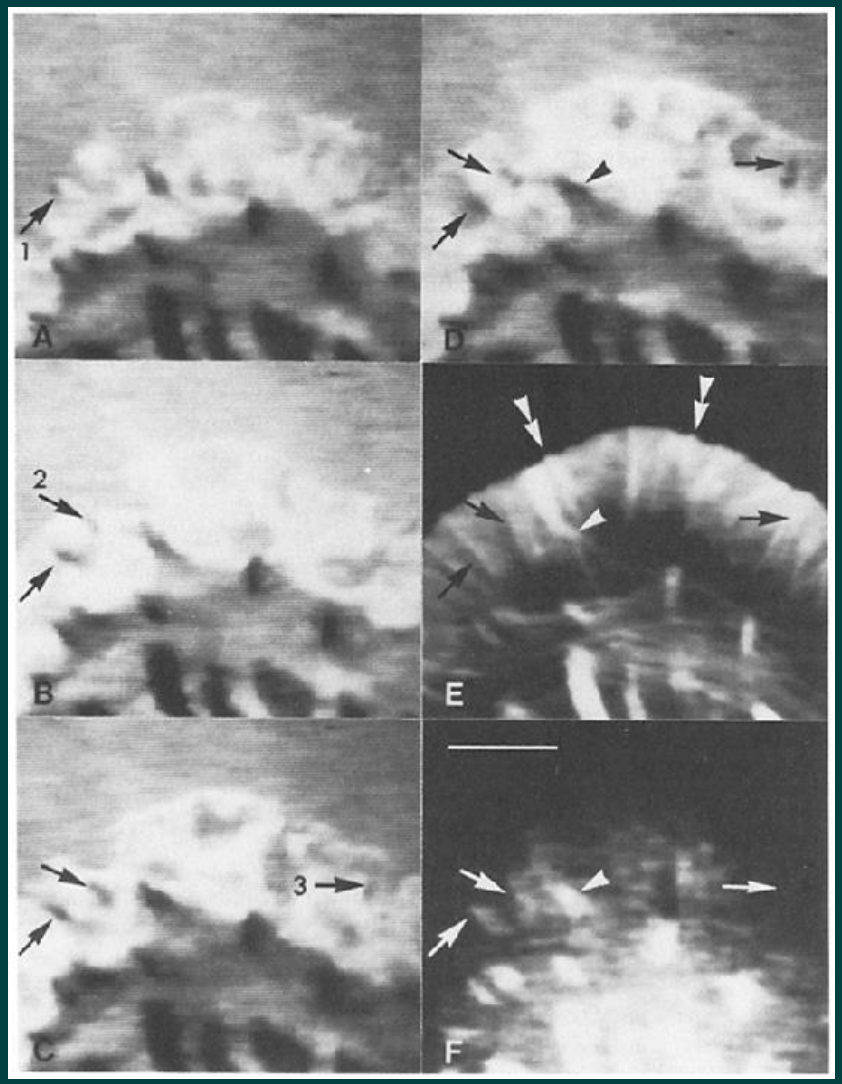
Fourniermet al., 2010

Cell adhesion

Clustering of actin and specific molecules at the sites where membrane is in close contact with the substrate

IRM

(interference-reflection microscopy)

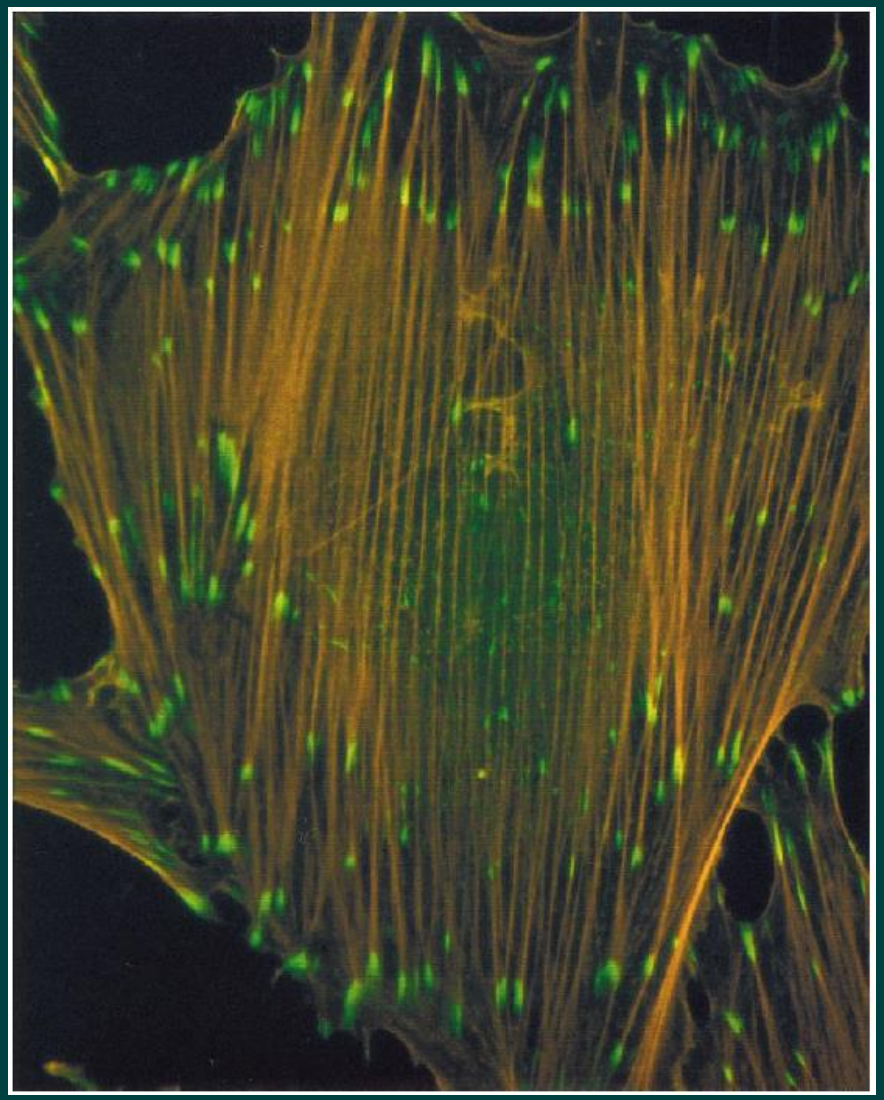


actin

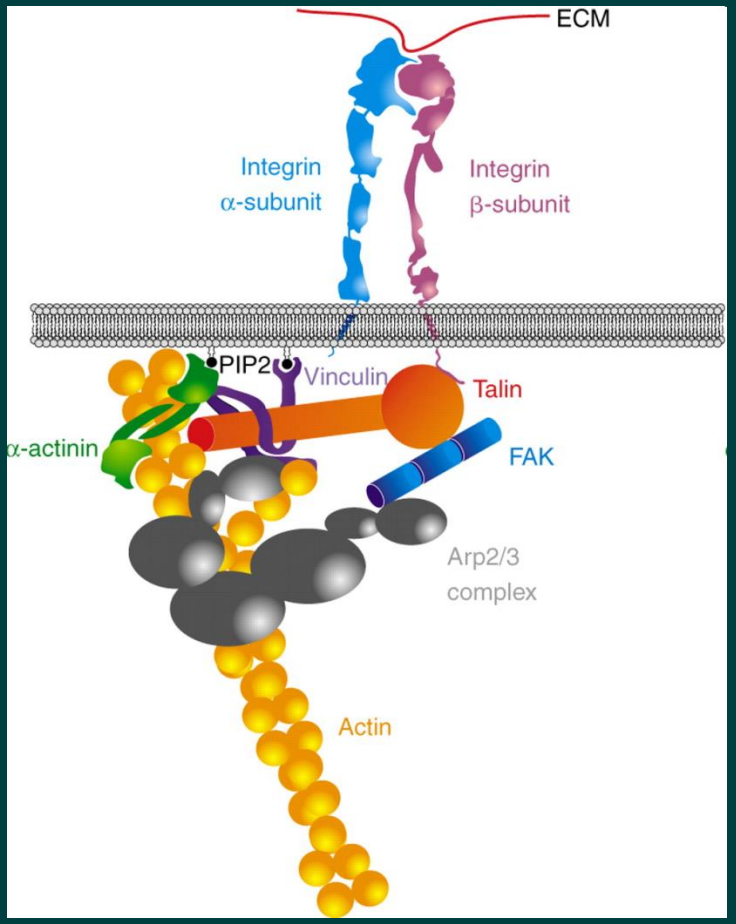
vinculin

DePasquale and Izzard, JCB, 1987

Focal adhesions connect actin filaments to the extracellular matrix

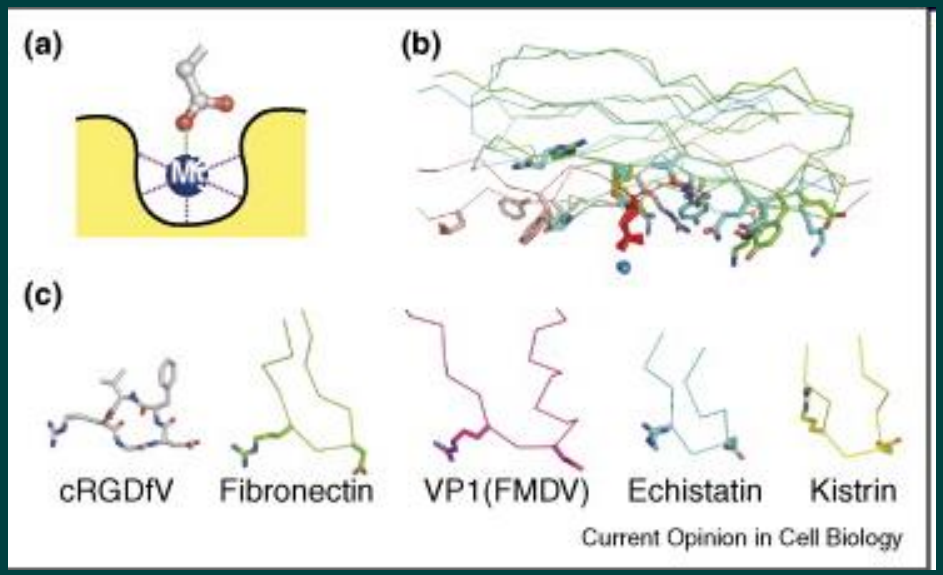


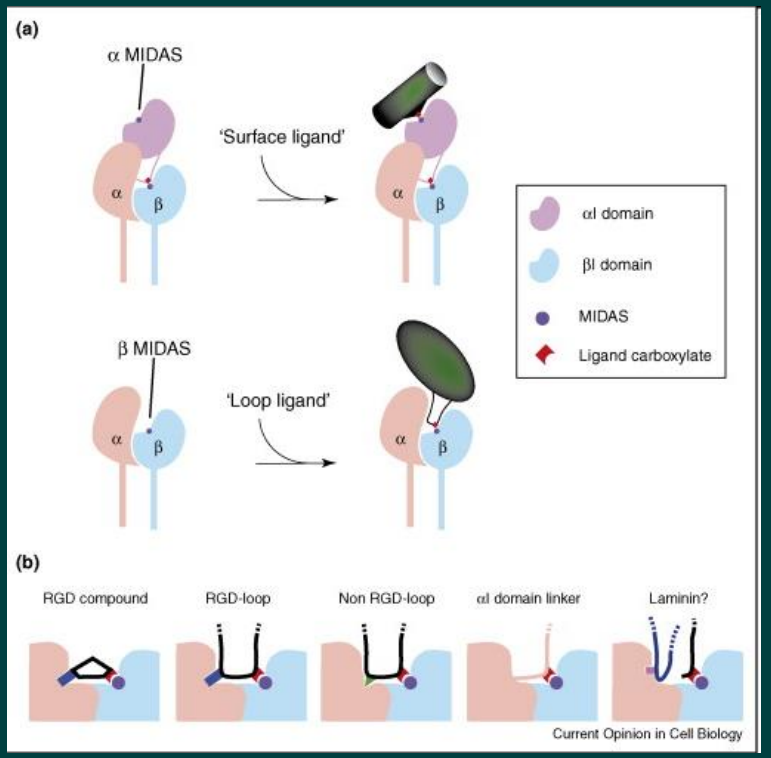
Sastry and Burridge, Exp. Cell Res., 2000



Vicente-Manzanares et al., J.Cell Sci., 2009

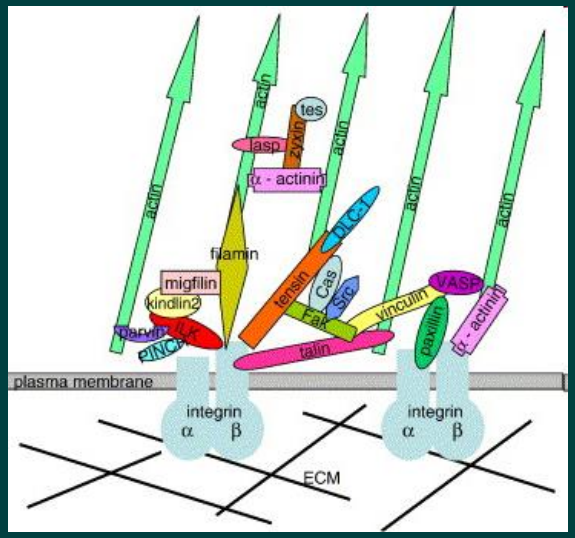
Integrins are heterodimers of α and β subunits (18 different alpha and 8 beta in humans) that recognize Arg-Gly-Asp (RGD) sequence in extracellular matrix proteins through metal-ion (e.g., Mg)-dependent adhesion site (MIDAS)



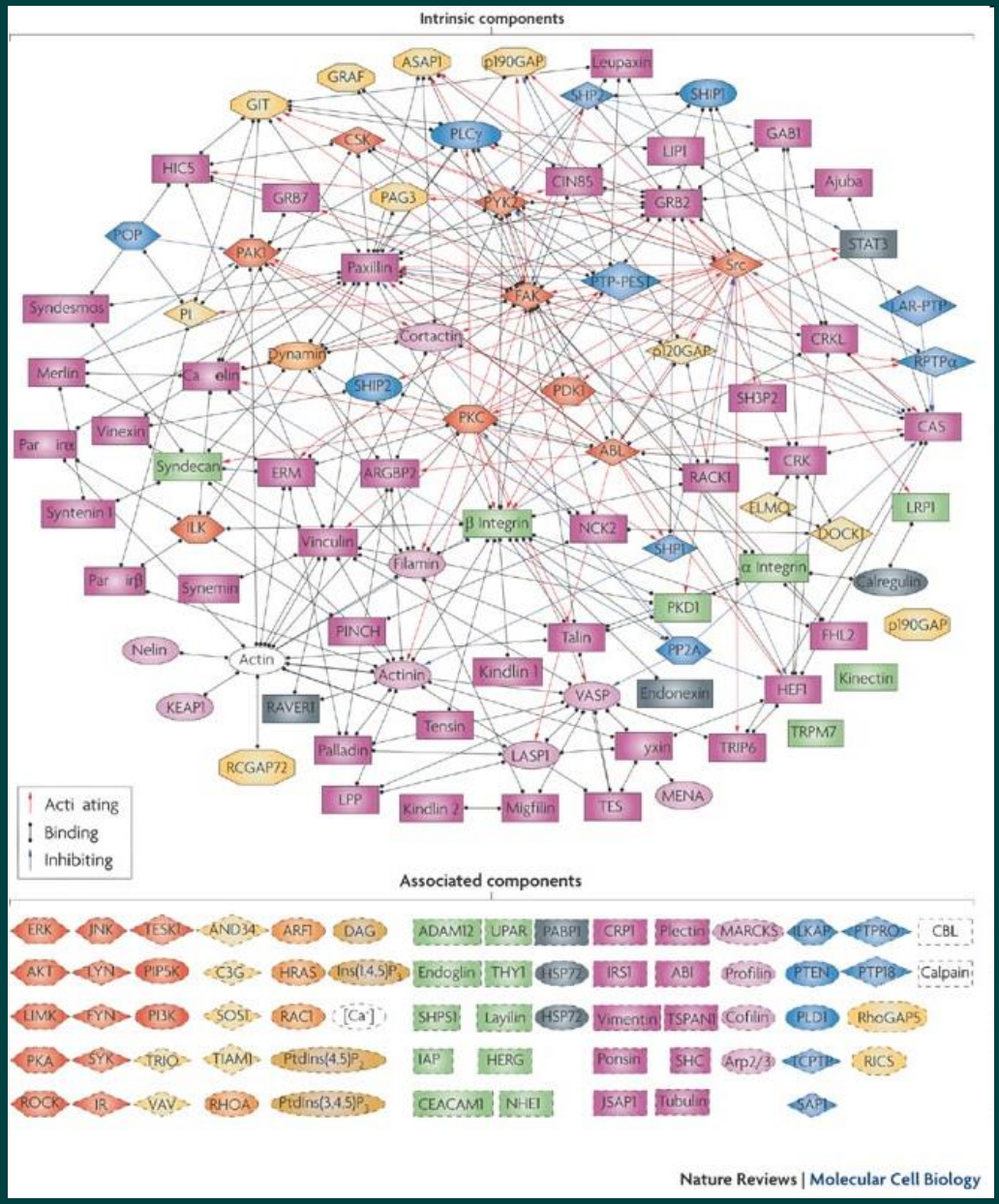


Takagi, Curr. Op. Cell Biol., 2007

Components of cell-matrix adhesions



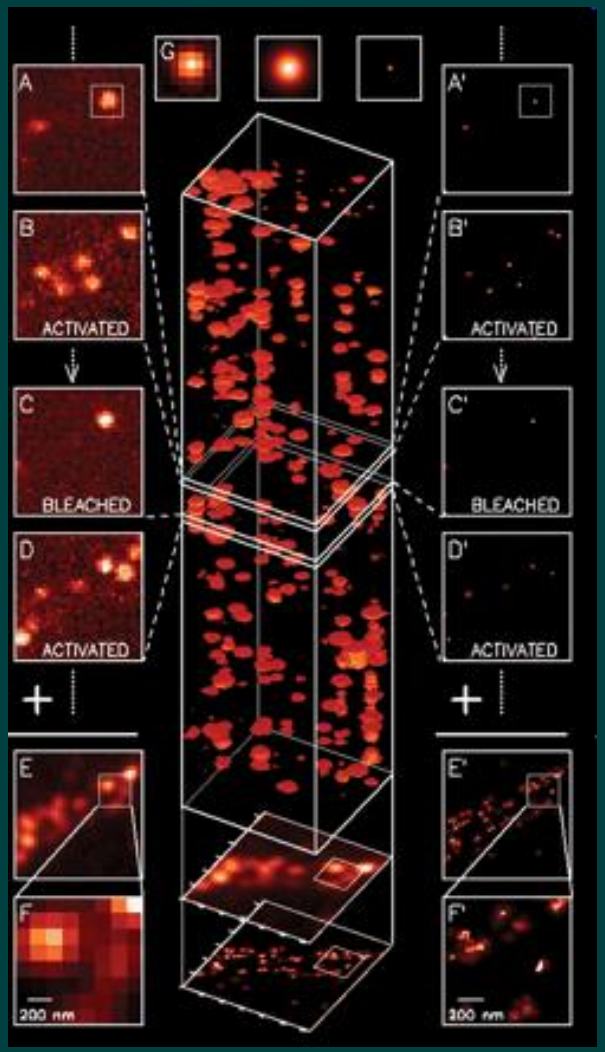
Lo, Dev. Biol., 2006

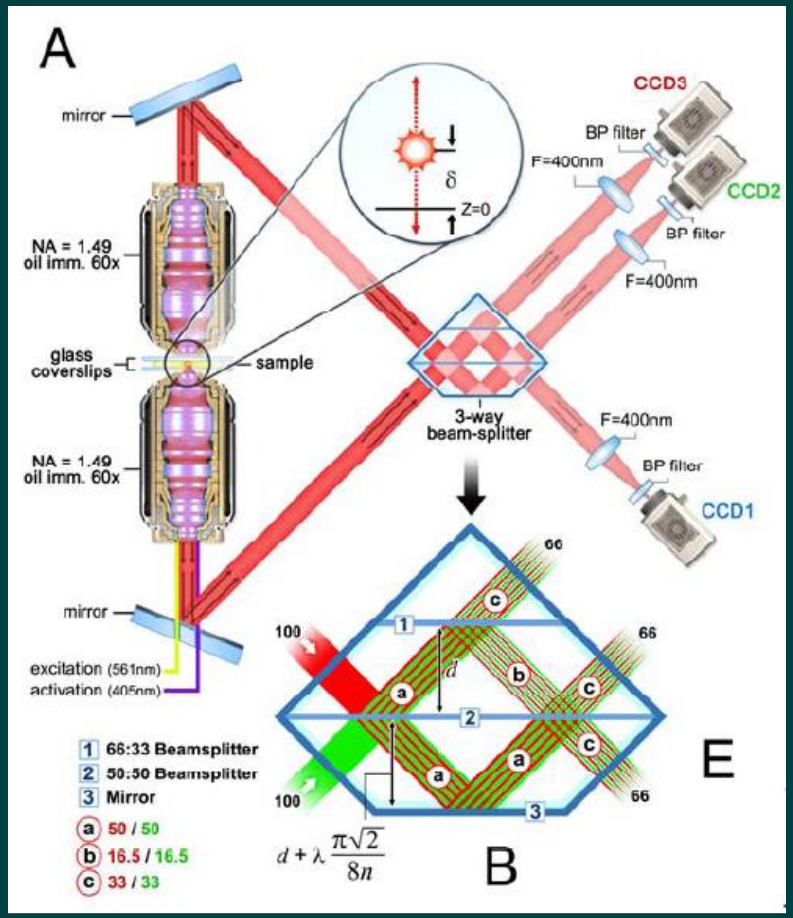


Geiger et al., 2009, Nature Reviews

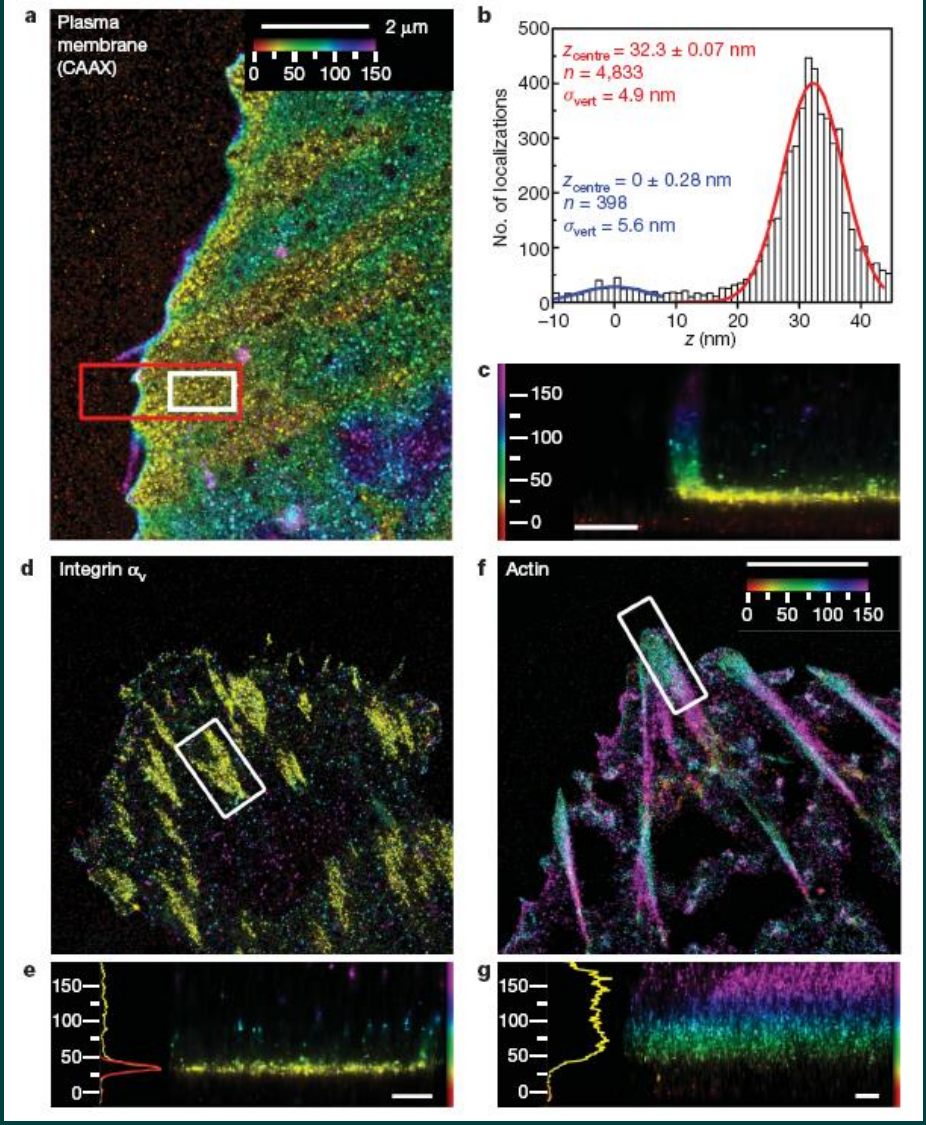
How are molecules connected to each other?
Molecular architecture of FA

iPALM (photoactvation localization microscopy with interferometry)



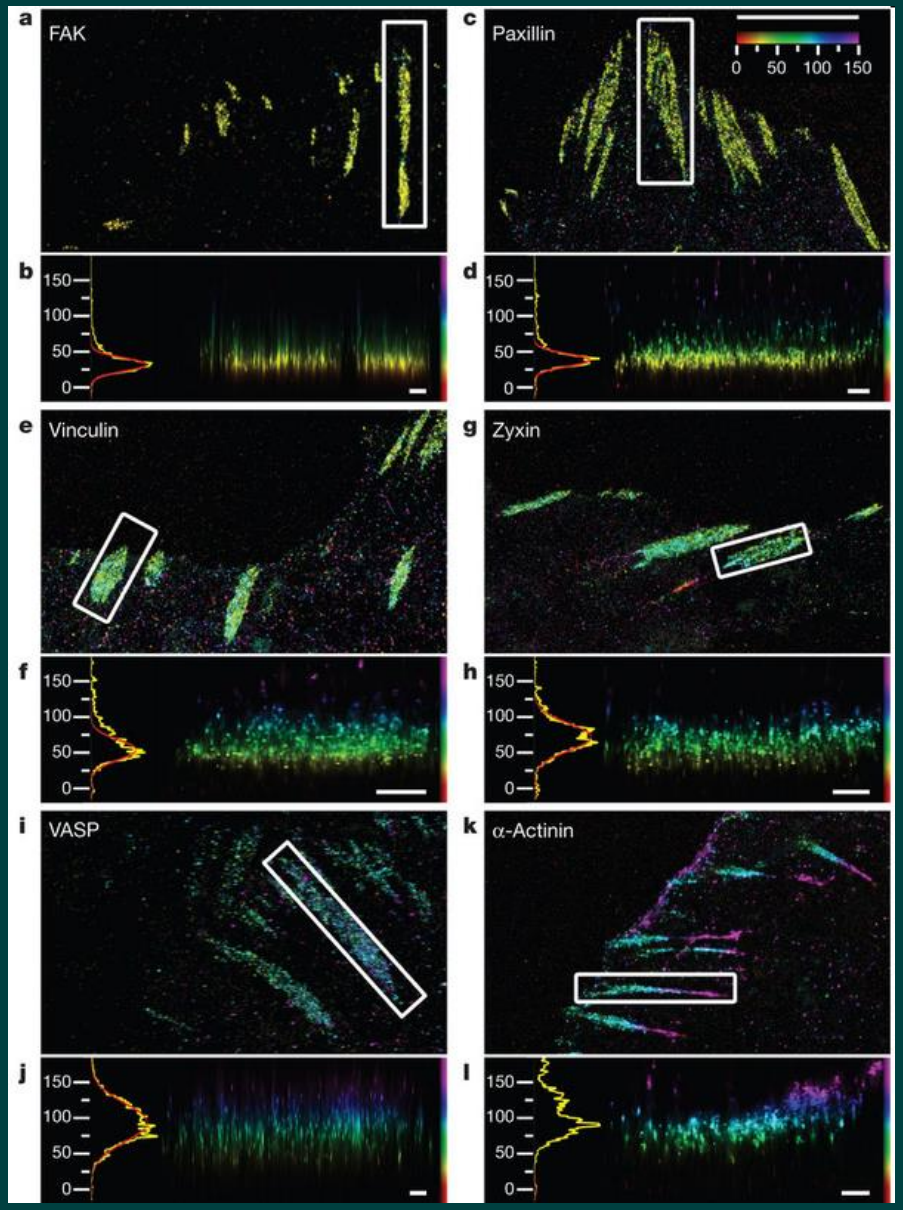


How are molecules connected to each other?
Molecular architecture of FA

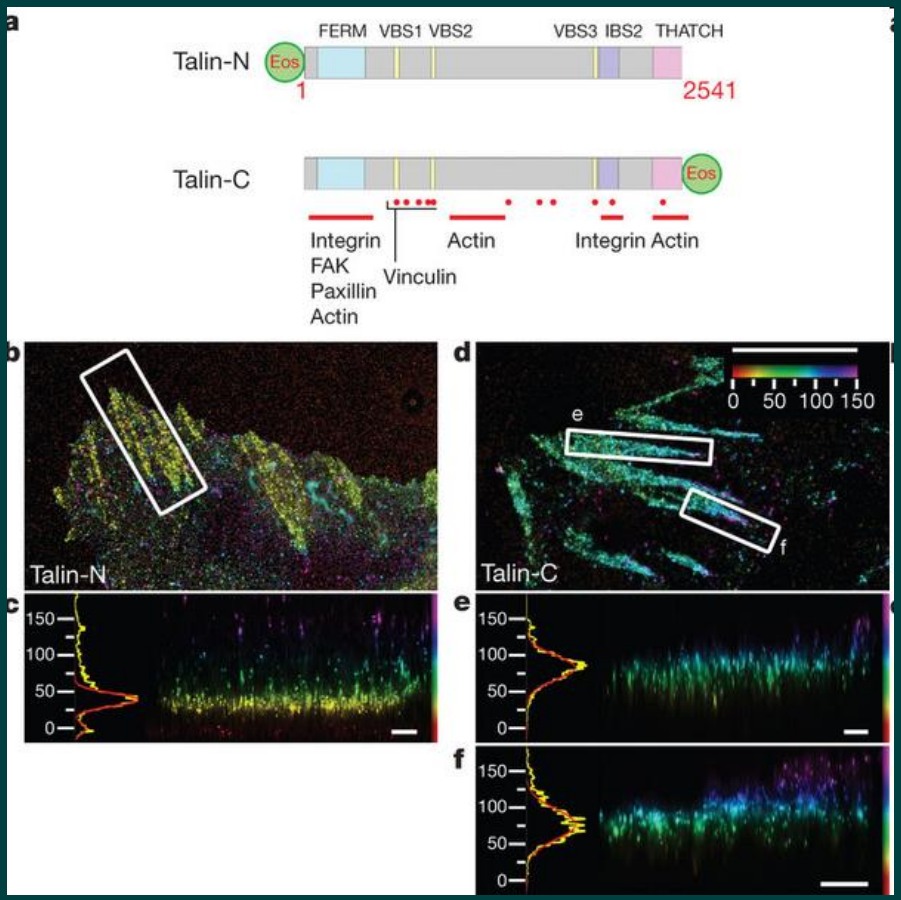


Kanchanawong et al., Nature, 2010

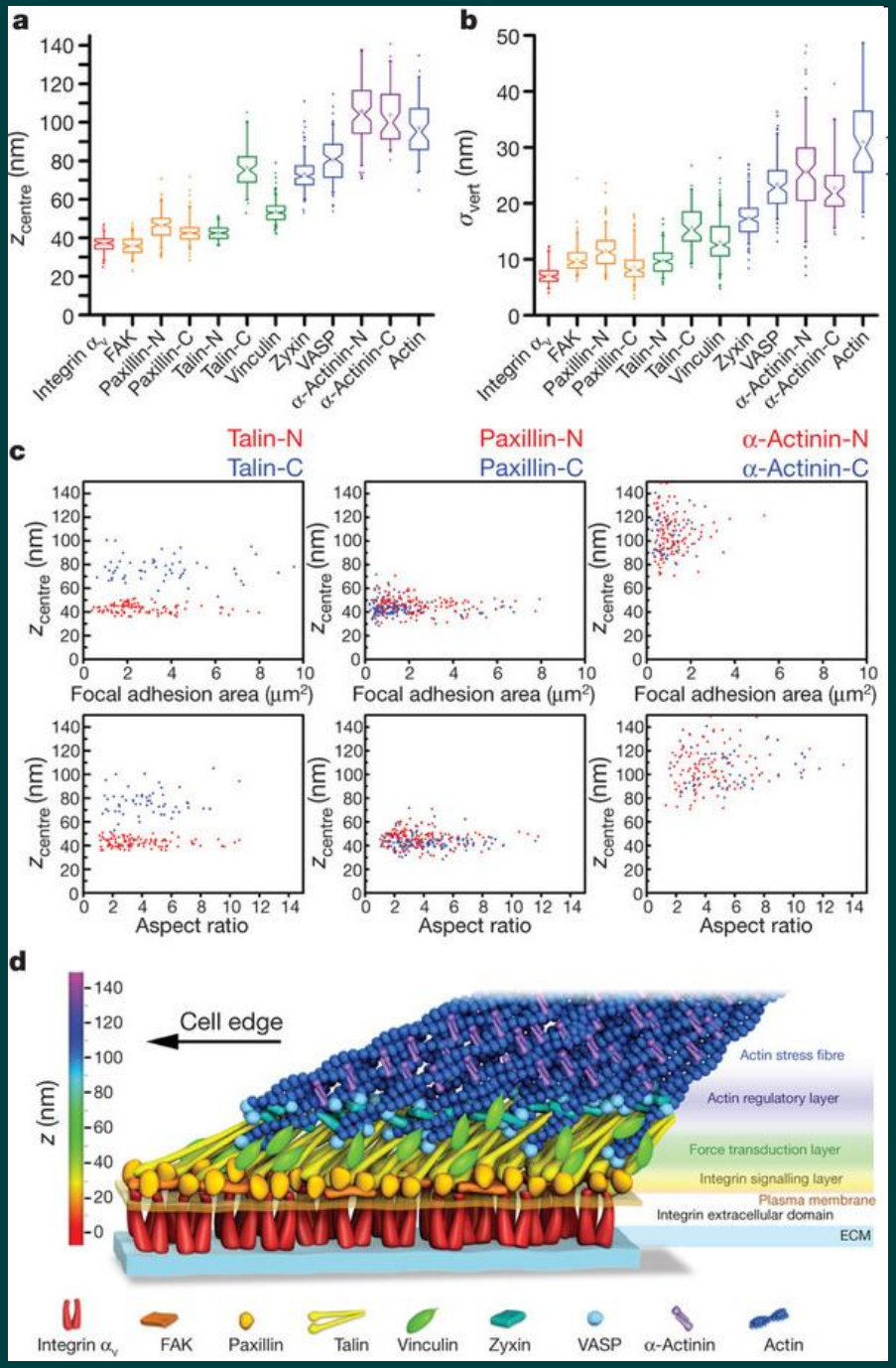
Relative positions of proteins



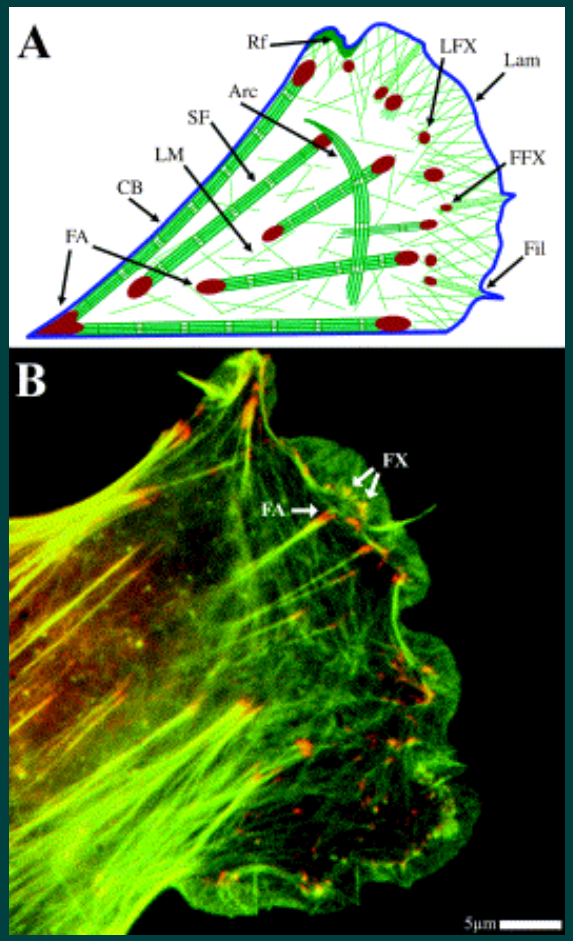
Talin orientation

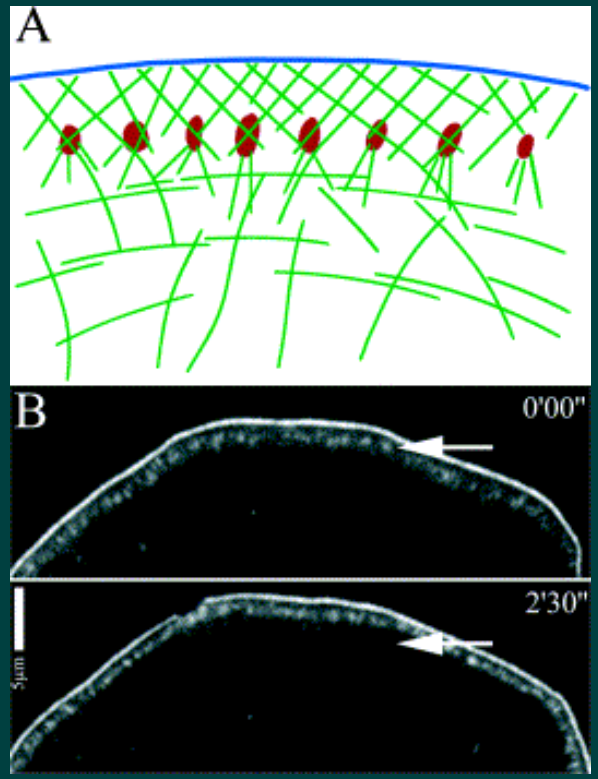


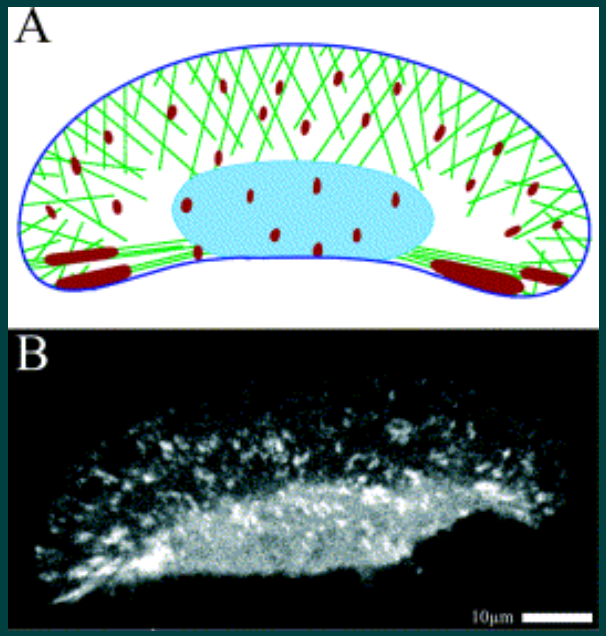
Kanchanawong et al., Nature, 2010


a
b
z_{centre} (nm)
σ_{vert} (nm)
Integrin α_v
FAK
Paxillin-N
Paxillin-C
Talin-N
Talin-C
Vinculin
Zyxin
VASP
α-Actinin-N
α-Actinin-C
Actin
c
Talin-N
Talin-C
Paxillin-N
Paxillin-C
α-Actinin-N
α-Actinin-C
Focal adhesion area (μm²)
Aspect ratio
d
z (nm)
Cell edge
Actin stress fibre
Actin regulatory layer
Force transduction layer
Integrin signalling layer
Plasma membrane
Integrin extracellular domain
ECM
Integrin α_v
FAK
Paxillin
Talin
Vinculin
Zyxin
VASP
α-Actinin
Actin

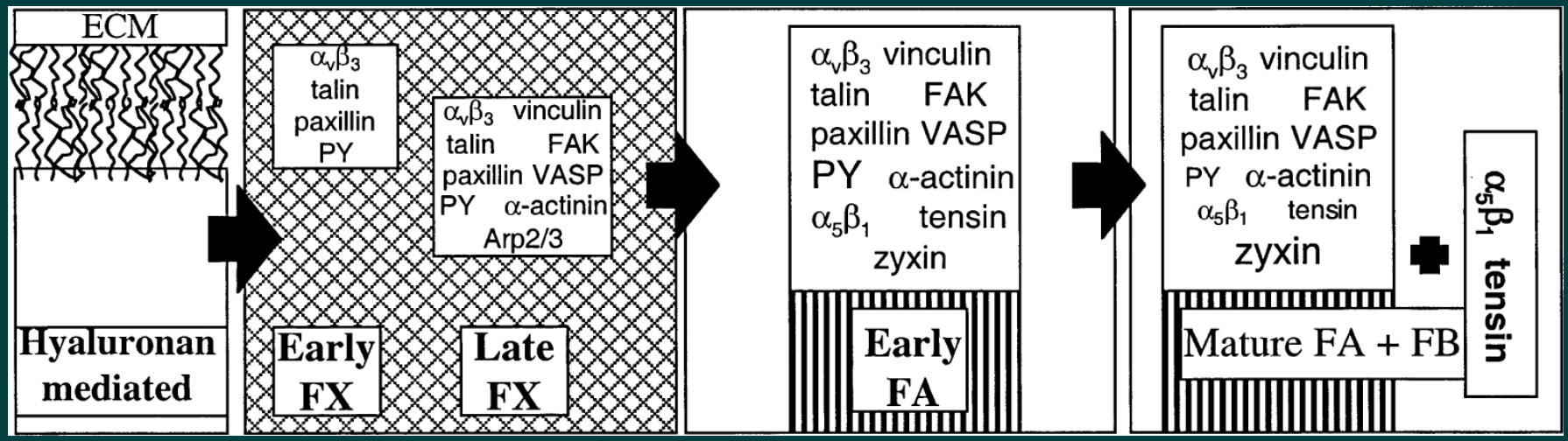
Different types of adhesion organization

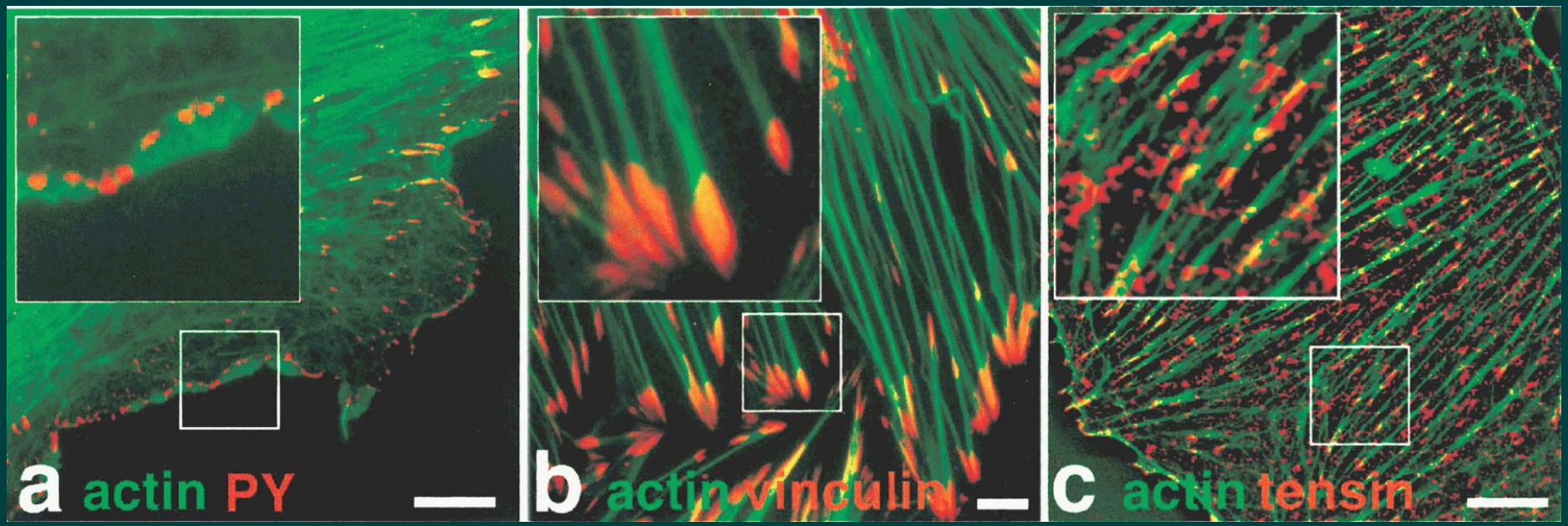






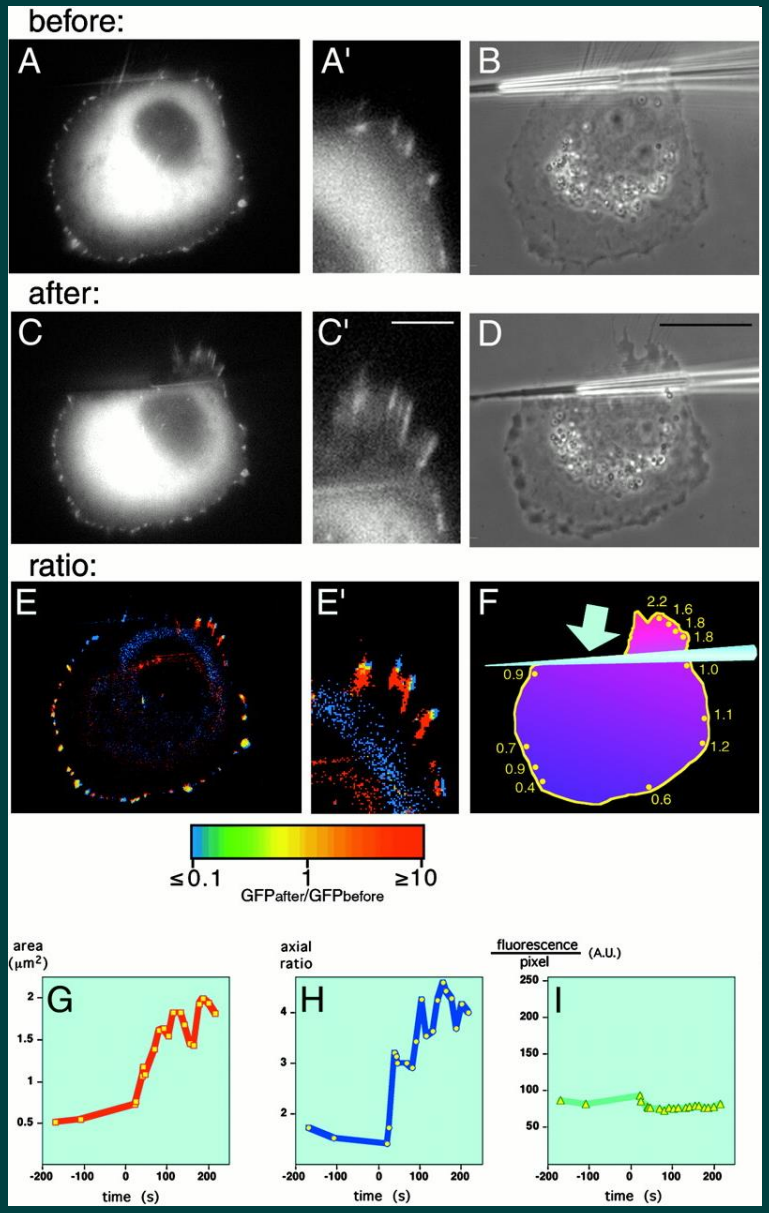
Adhesion maturation: focal complexes to mature focal adhesions (FA)



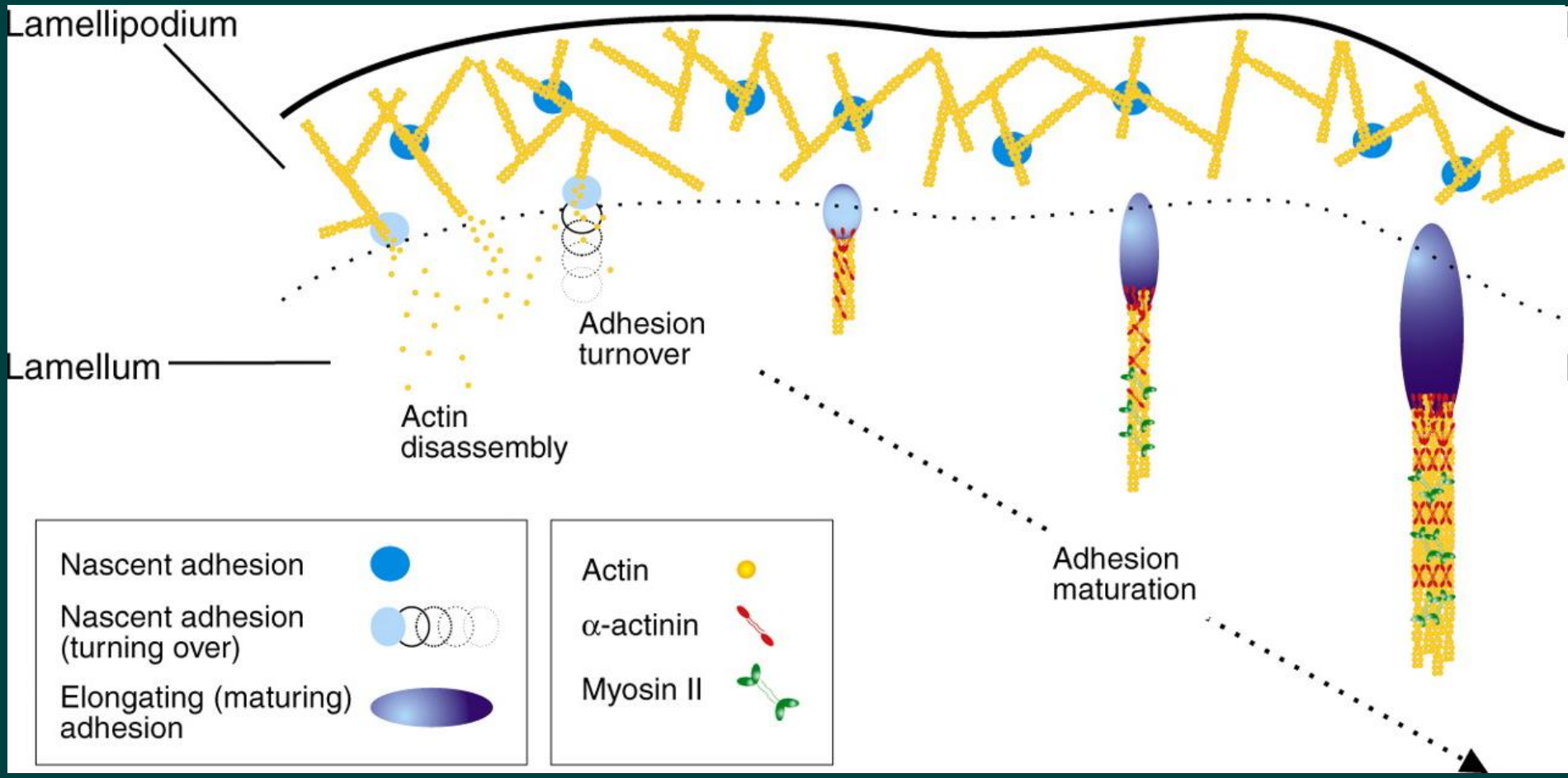


Zaidel-Bar et al., Biochem. Soc. Trans.,2004

FA maturation depends on force: internal (myosin II-dependent), or external

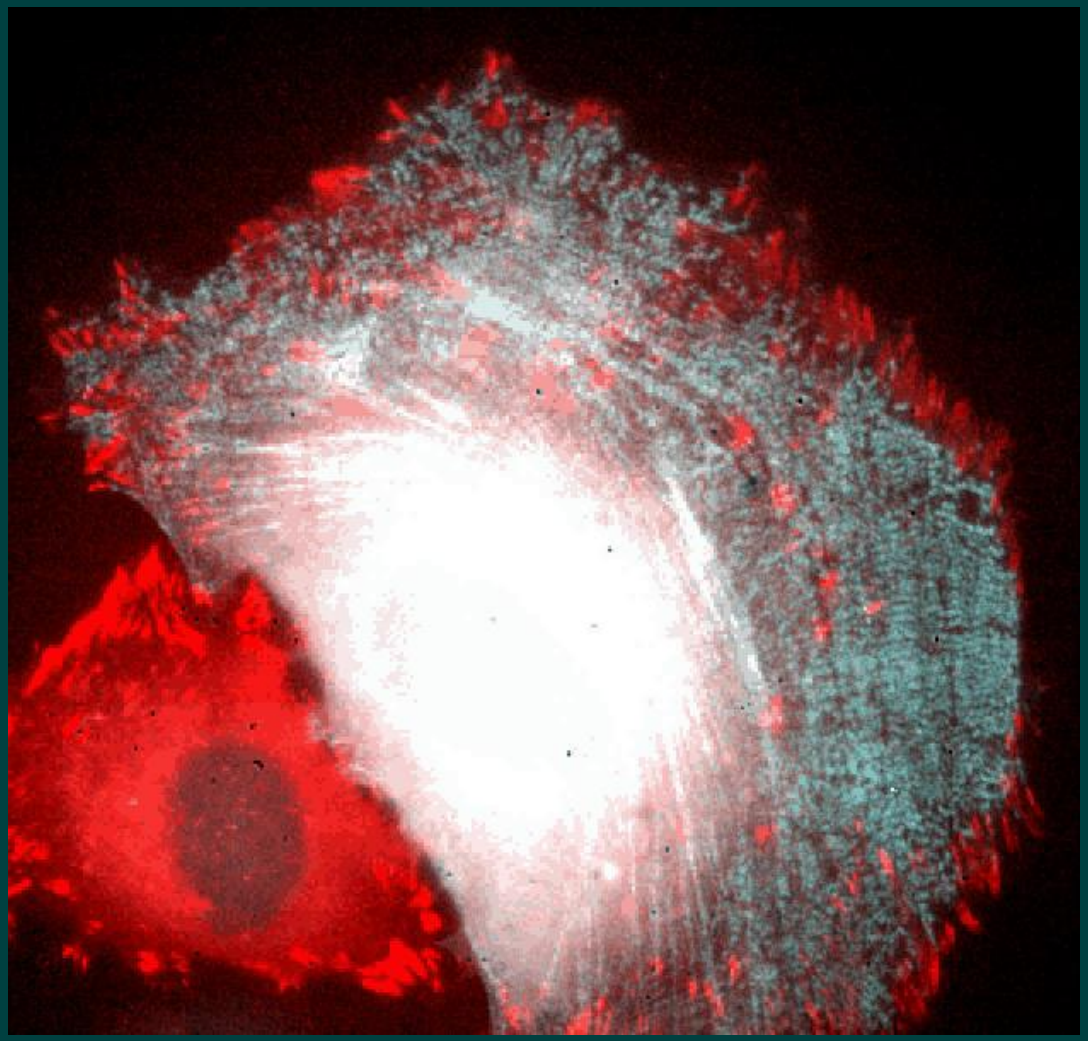


Riveline et al., JCB, 2001



Vicente-Manzanares et al., J.Cell Sci., 2009

Stress fibers contract and slide with respect to stationary adhesions



nest time: mechanics of cytoskeleton-adhesion connection
motion without specific adhesion
more about polarization mechanisms