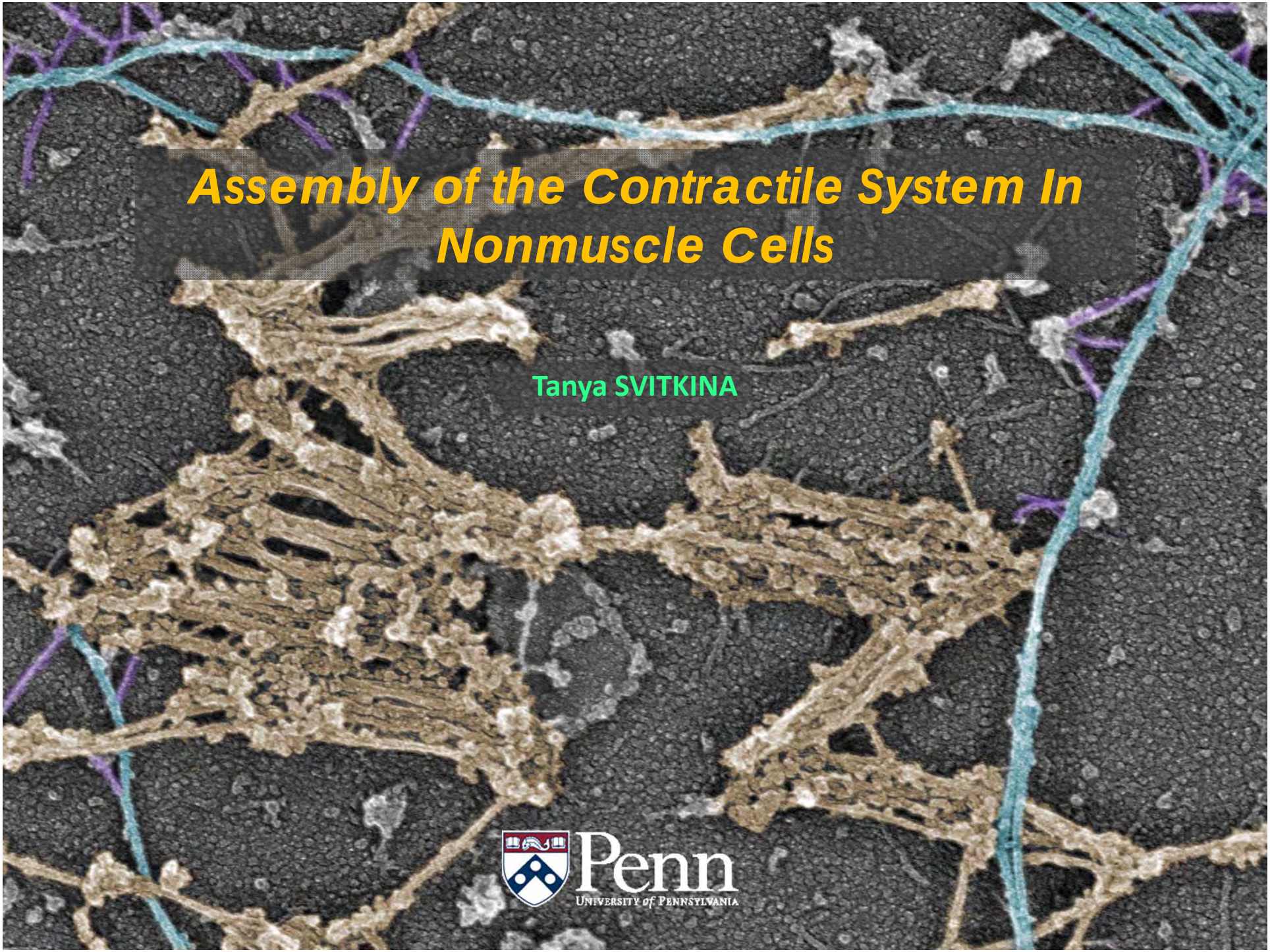
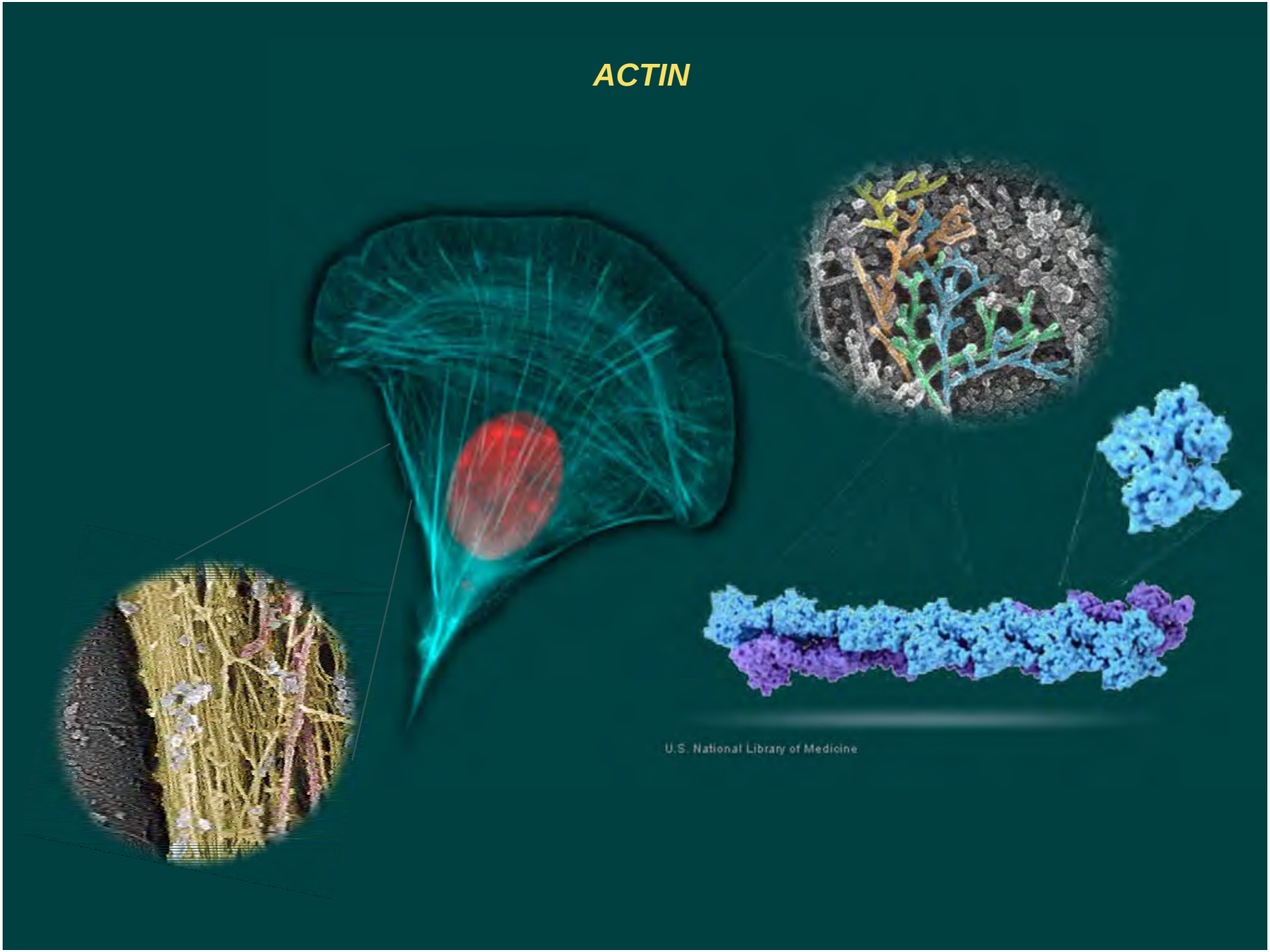
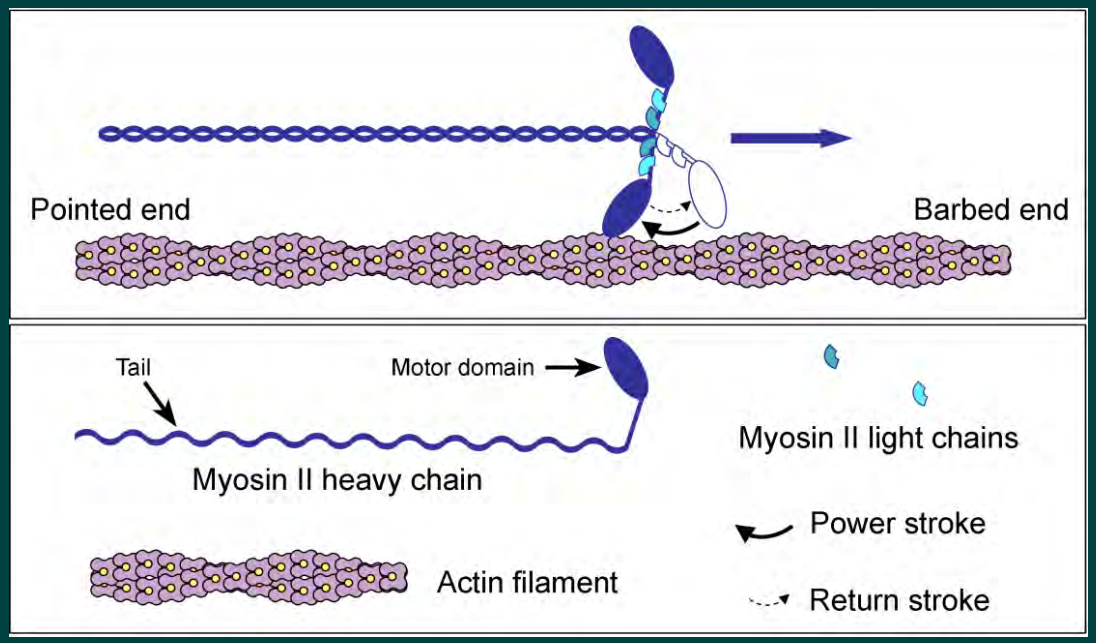
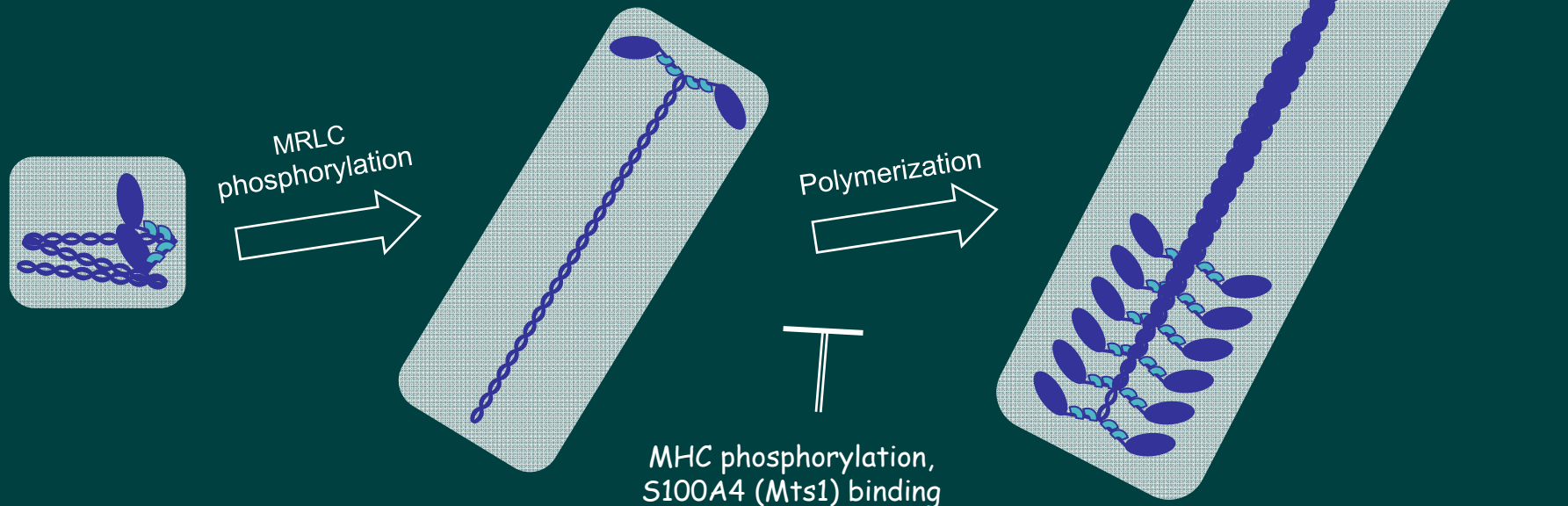

Assembly of the Contractile System In Nonmuscle Cells
Tanya SVITKINA
Penn
University of Pennsylvania

ACTIN

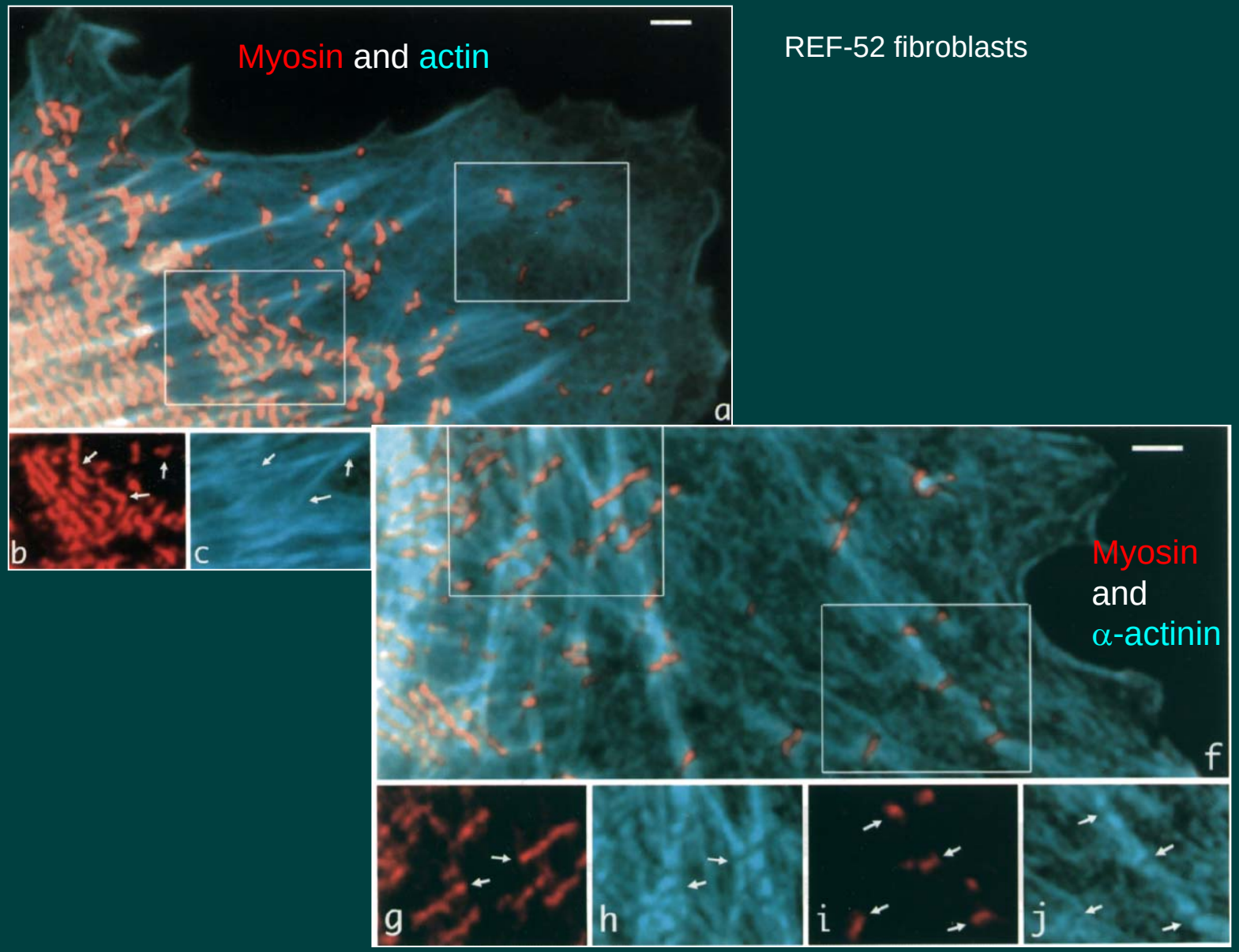


MYOSIN II


Pointed end
Barbed end
Tail
Motor domain
Myosin II heavy chain
Actin filament
Myosin II light chains
Power stroke
Return stroke


MRLC
phosphorylation
Polymerization
MHC phosphorylation,
S100A4 (Mts1) binding

QUASI-SARCOMERIC ORGANIZATION OF STRESS FIBERS

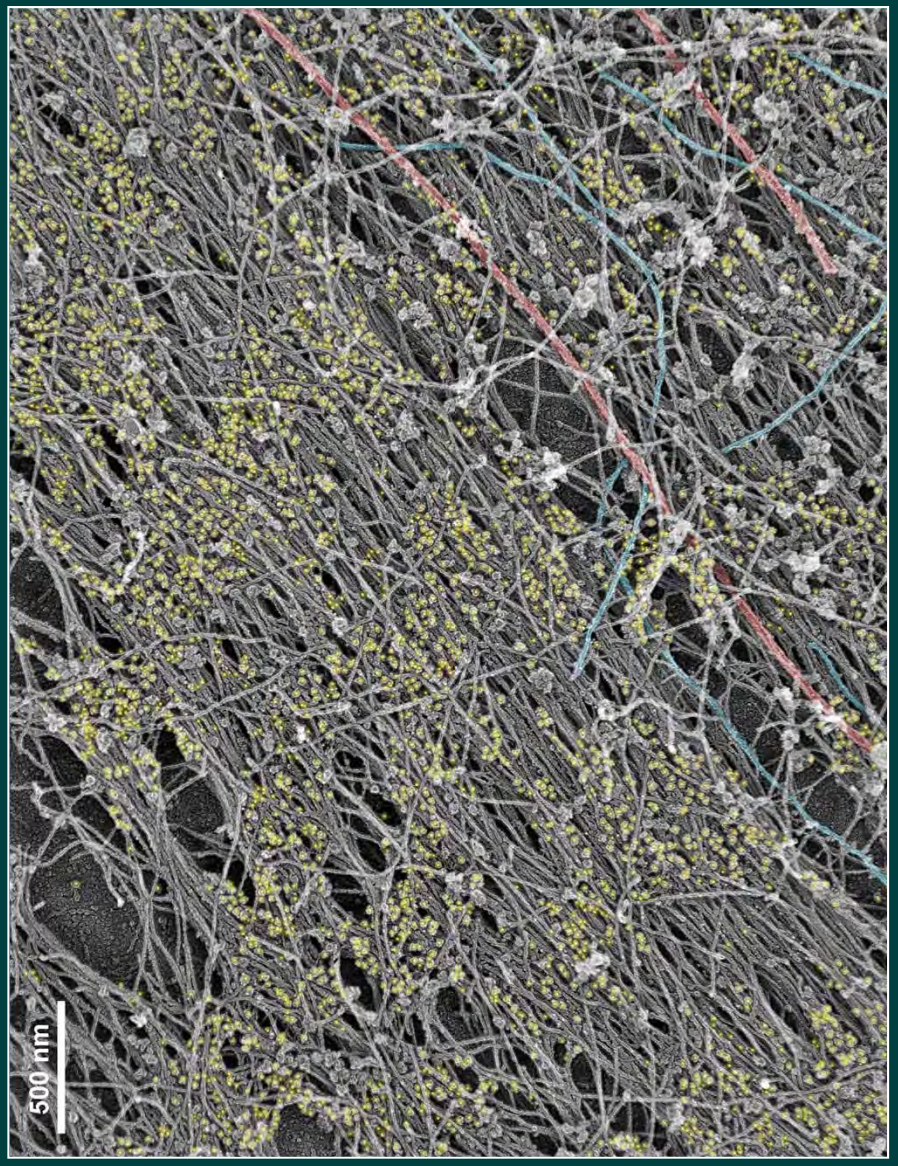


REF-52 fibroblasts

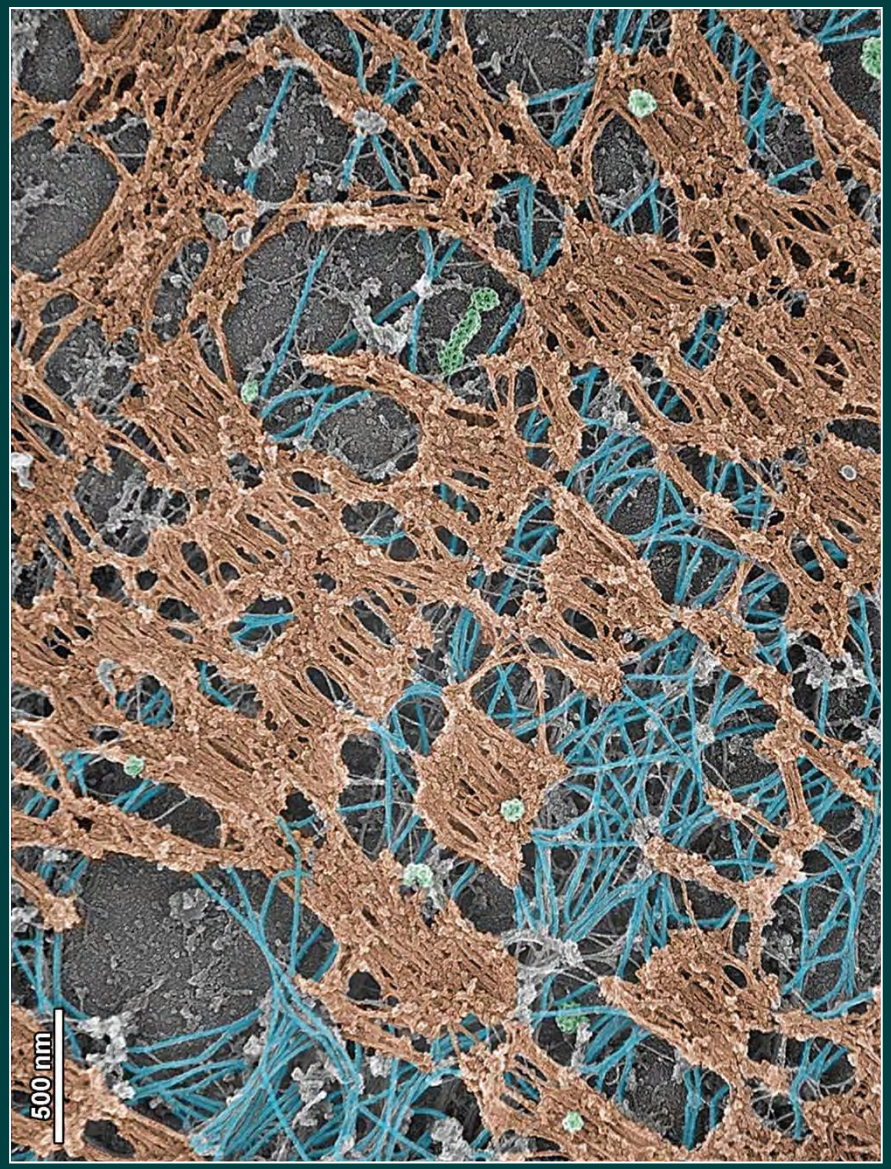
Verkhovsky et al., 1995

MYOSIN II IN STRESS FIBERS OF NONMUSCLE CELLS

Myosin II labeled with gold-conjugated antibody

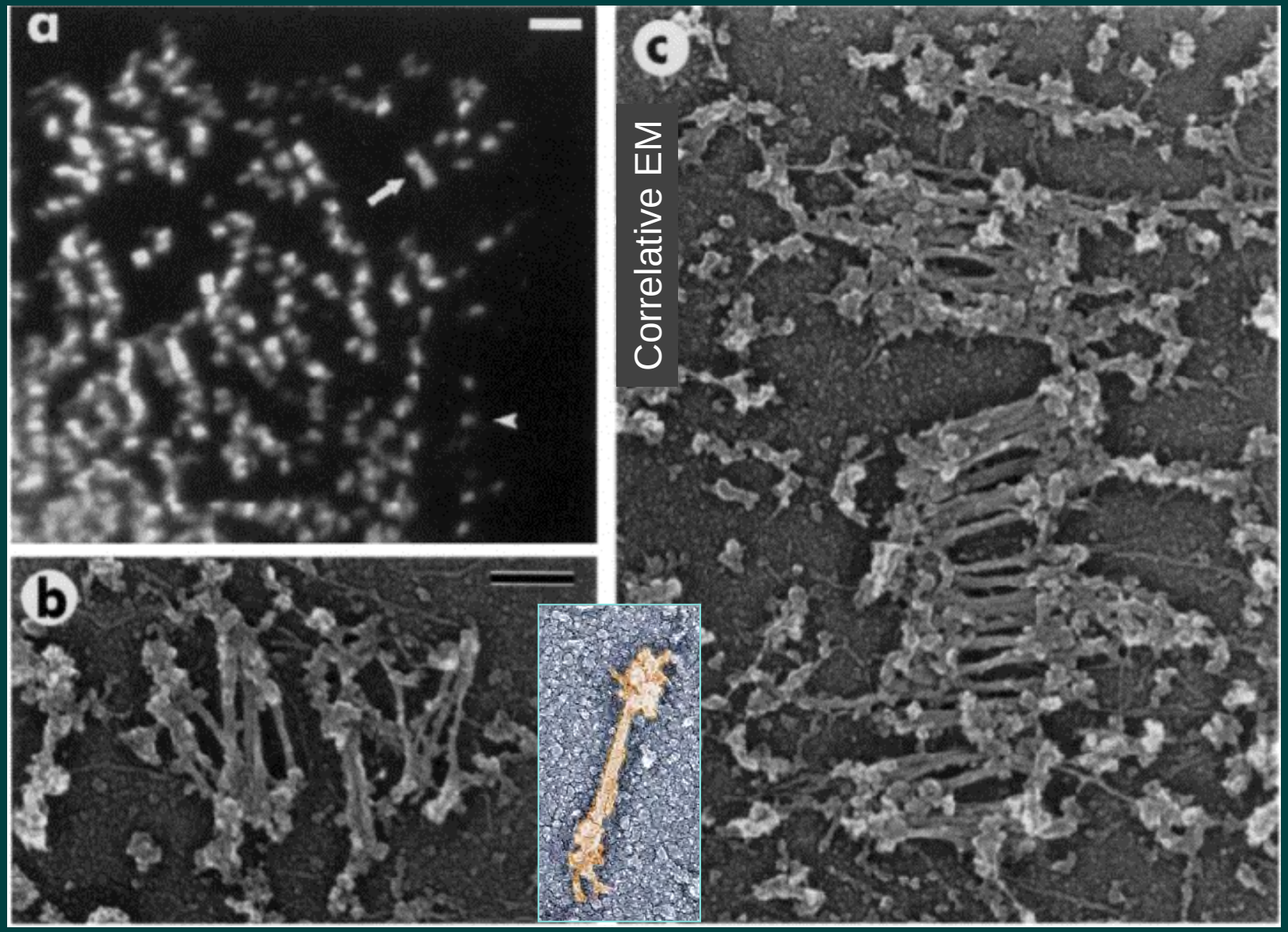


Myosin II filaments in the absence of actin



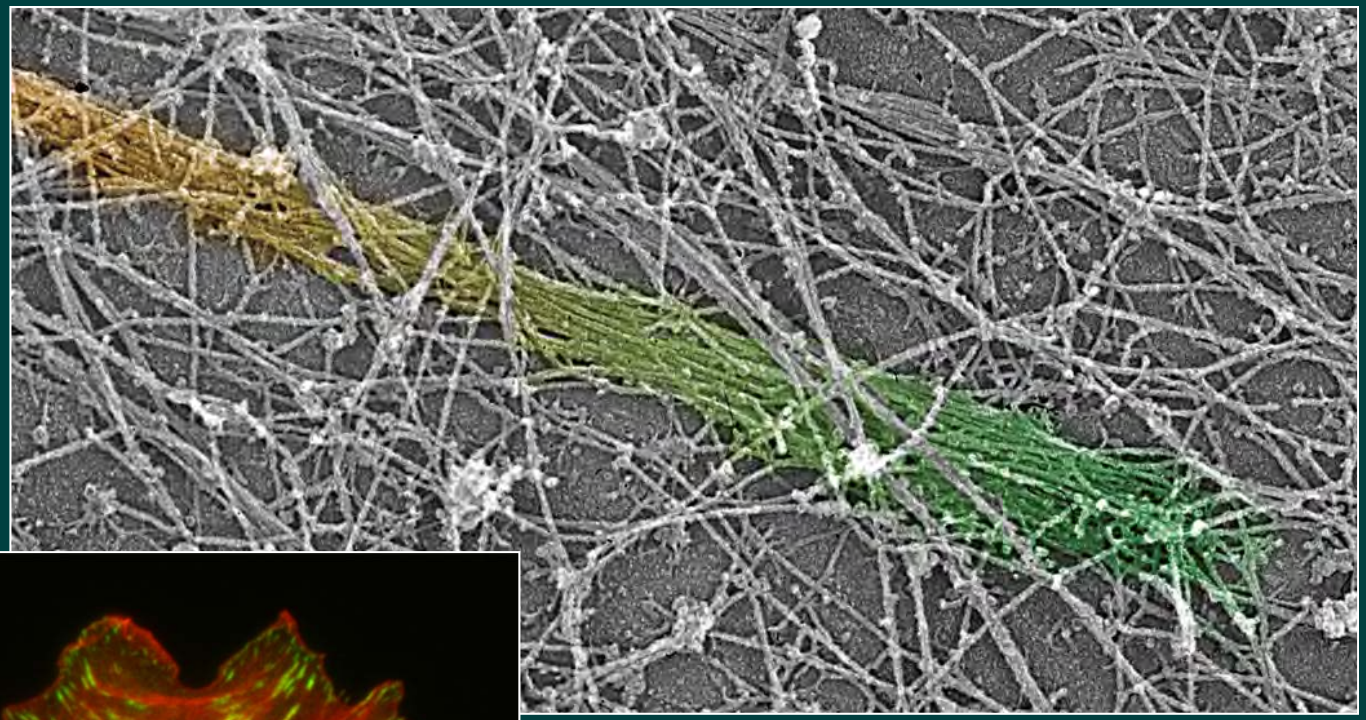
Verkhovsky et al., 1995

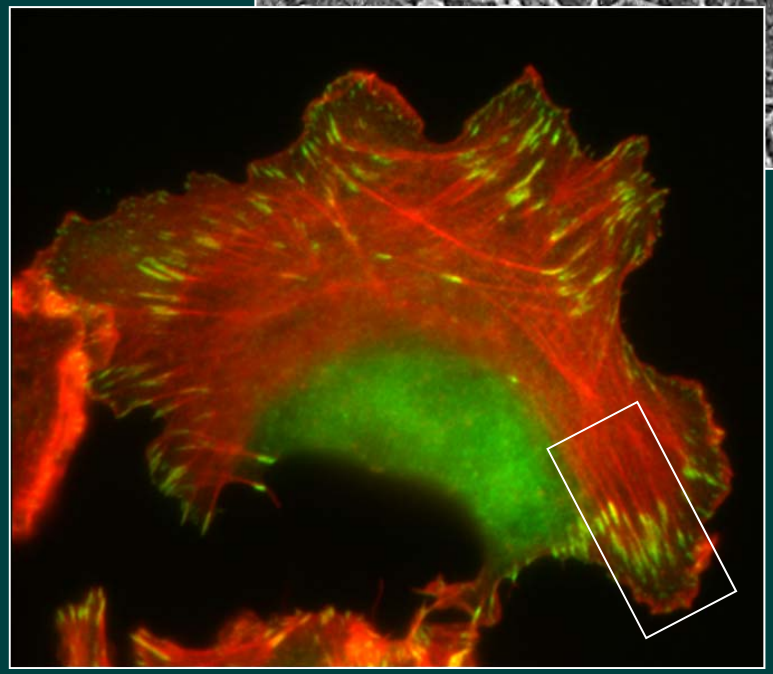
MYOSIN II IN LAMELLA OF NONMUSCLE CELLS



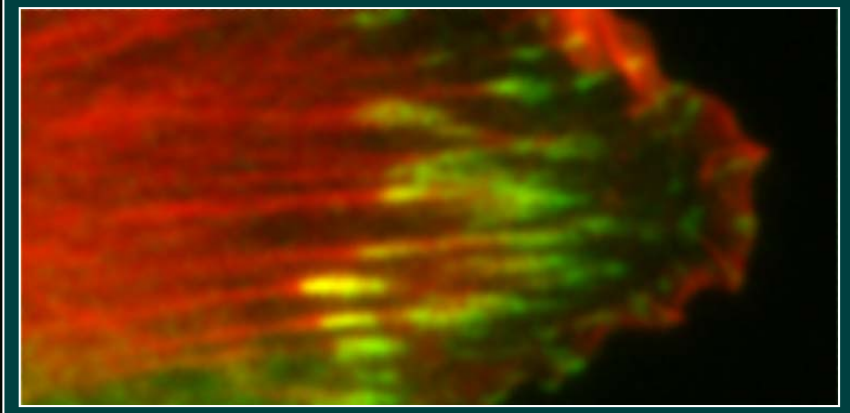
Verkhovsky et al., 1995

FOCAL ADHESIONS





Actin + vinculin



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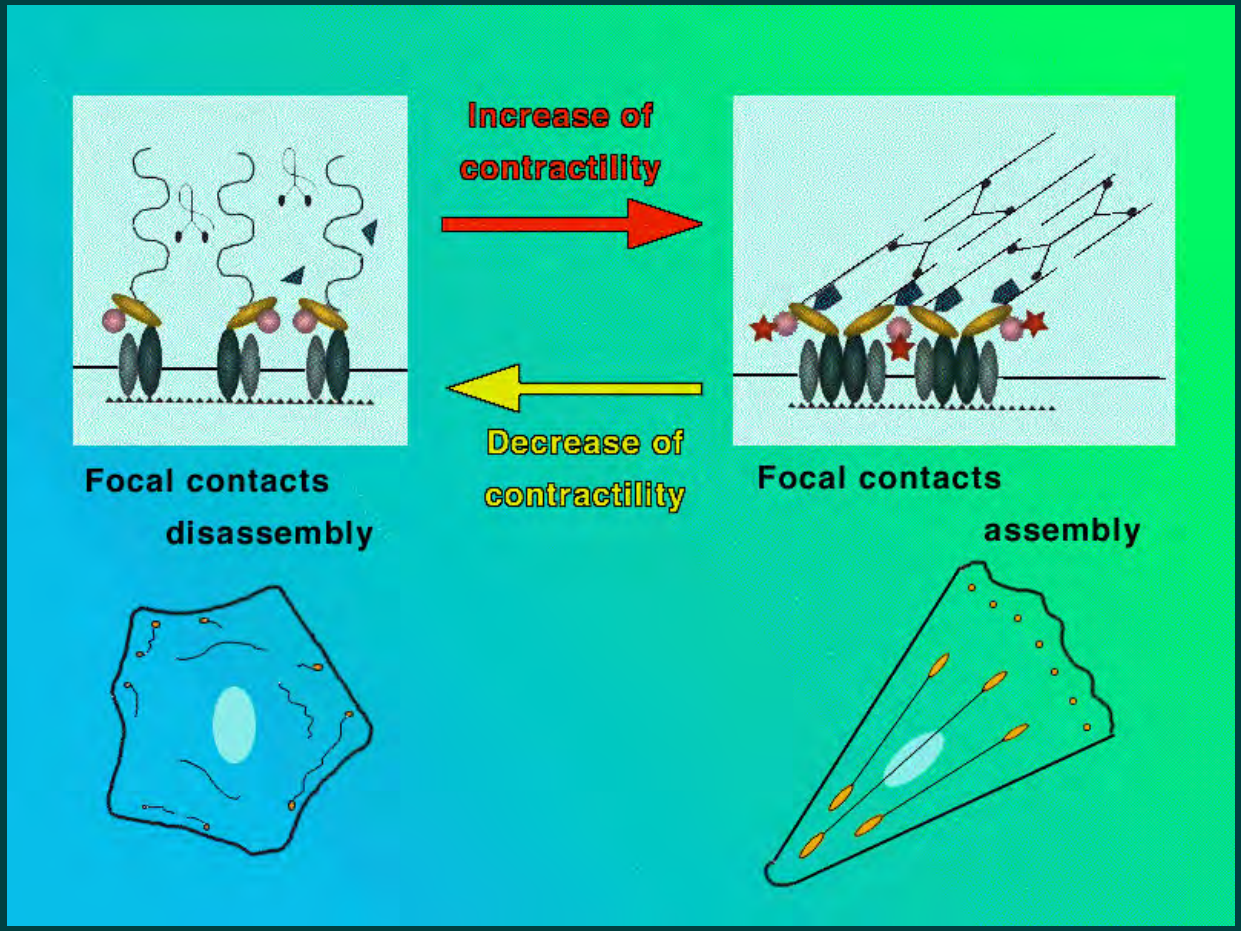
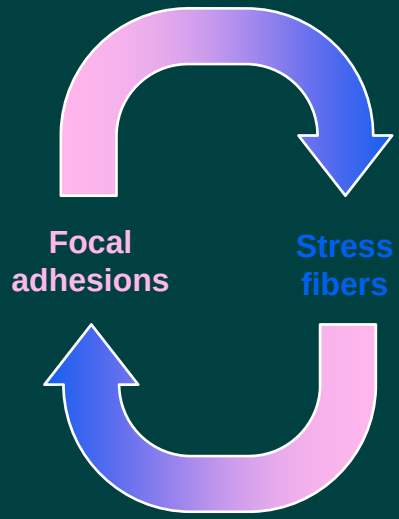
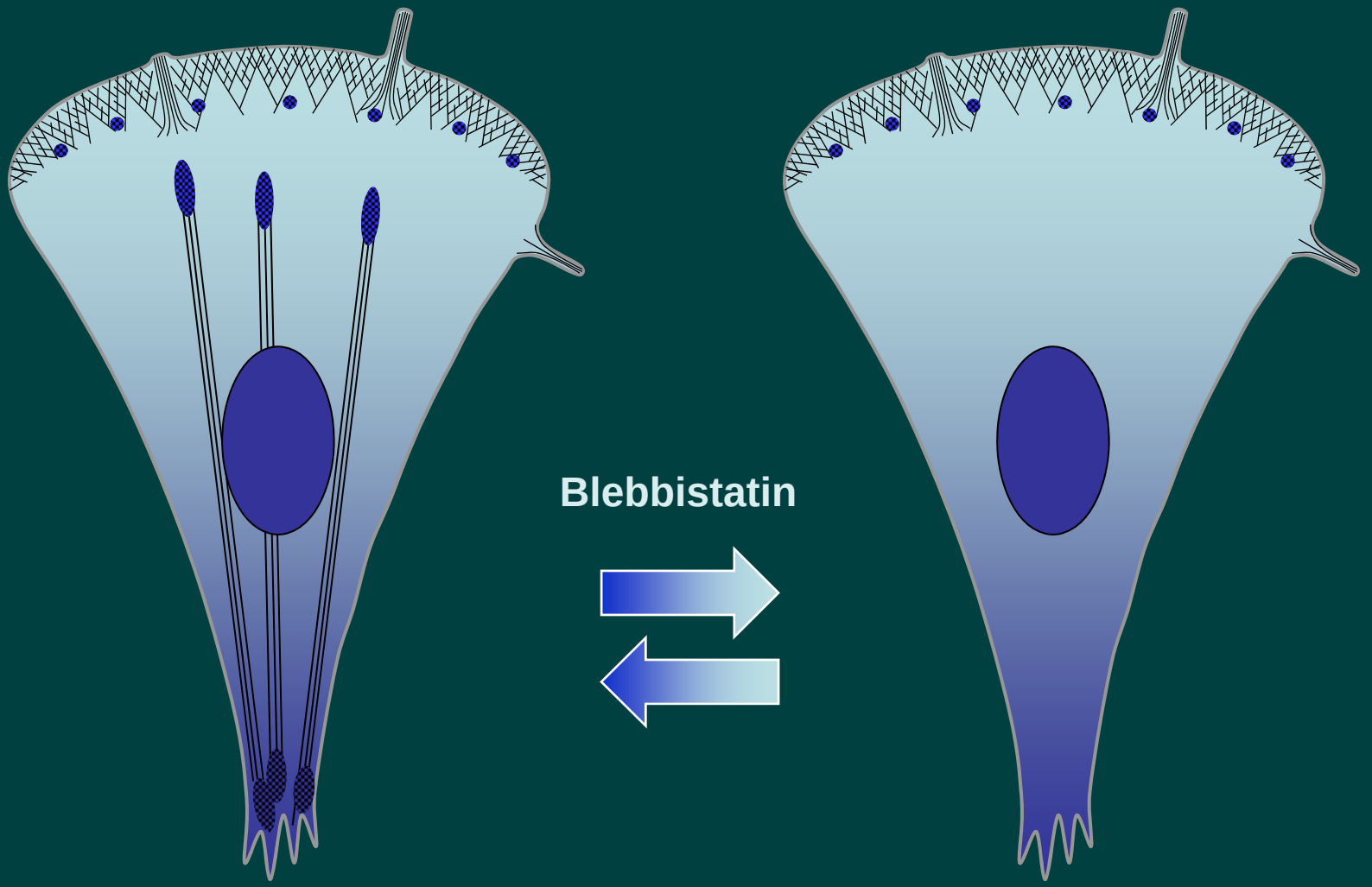
FORCE DEPENDANCE OF FOCAL ADHESION FORMATION





Burridge et al., 1997

HOW TO INVESTIGATE THE CONTRACTILE SYSTEM ASSEMBLY?

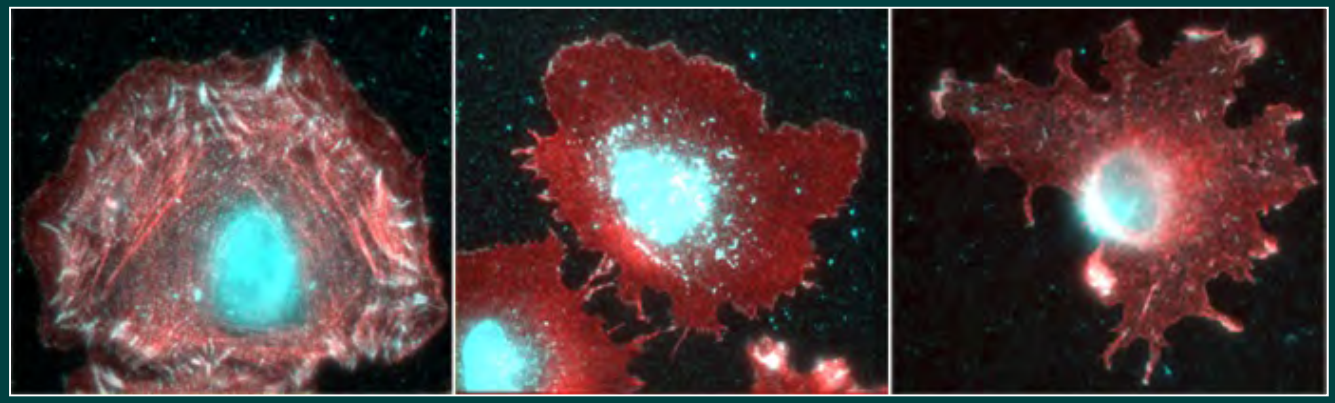


LAMELLIPODIA DEPEND ON MYOSIN II ACTIVITY

Blebbistatin treatment

Control | 75 μM | 100 μM

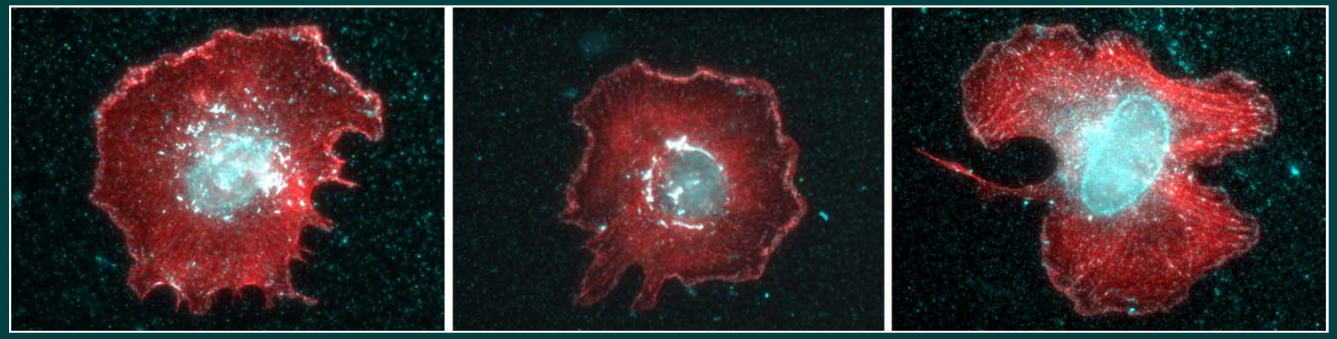
F-actin + α-actinin



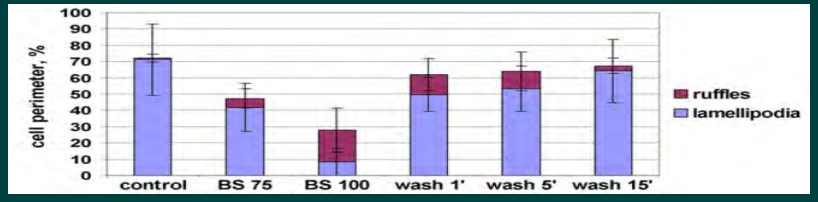
Washout of 100 μM blebbistatin

1 min | 5 min | 15 min

F-actin + α-actinin



Lamellipodia inhibition and recovery



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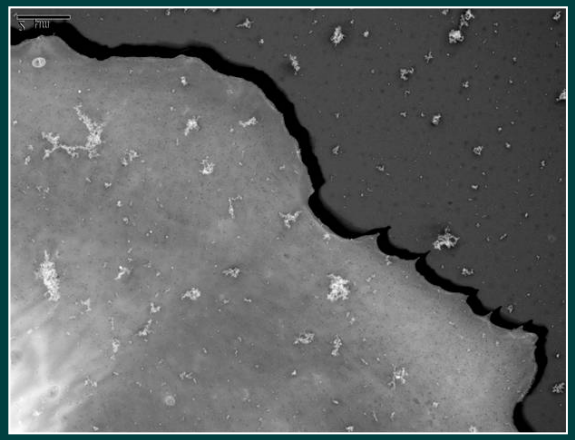
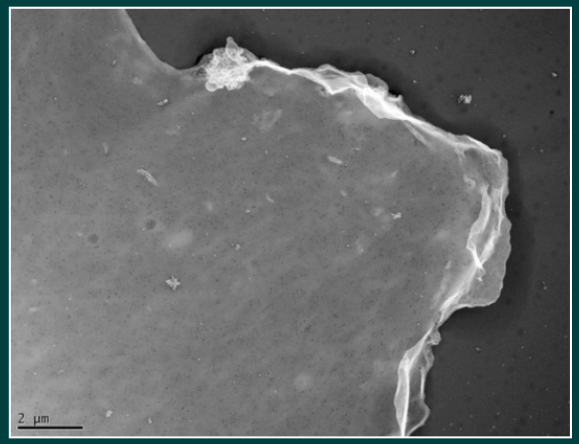
LAMELLIPODIA DEPEND ON MYOSIN II ACTIVITY (EM)

Blebbistatin treatment

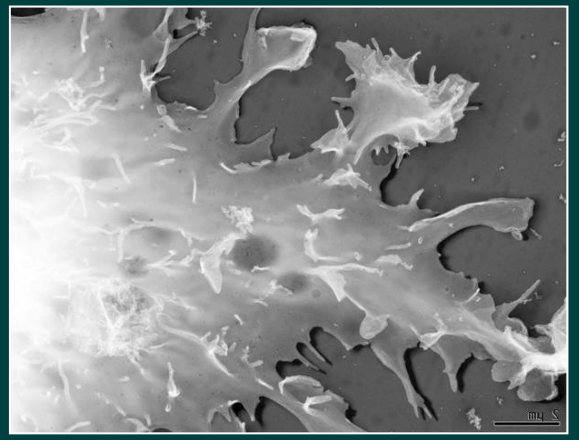
Control



75 μM

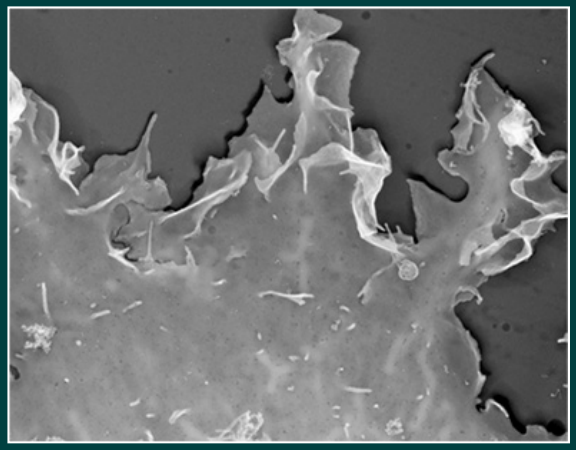


100 μM

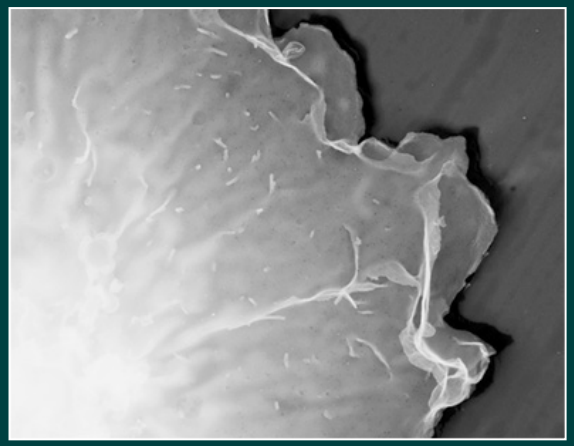


Washout of 100 μM blebbistatin

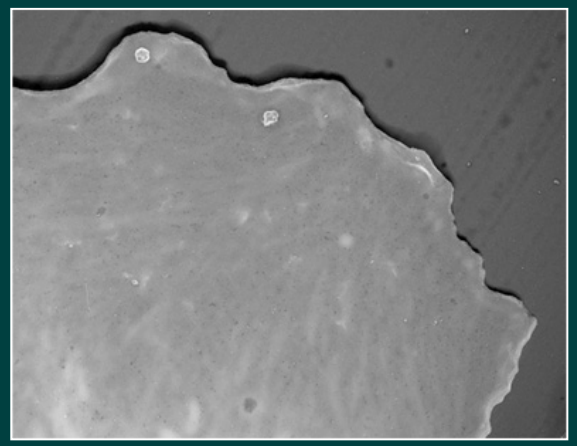
1 min



5 min



15 min



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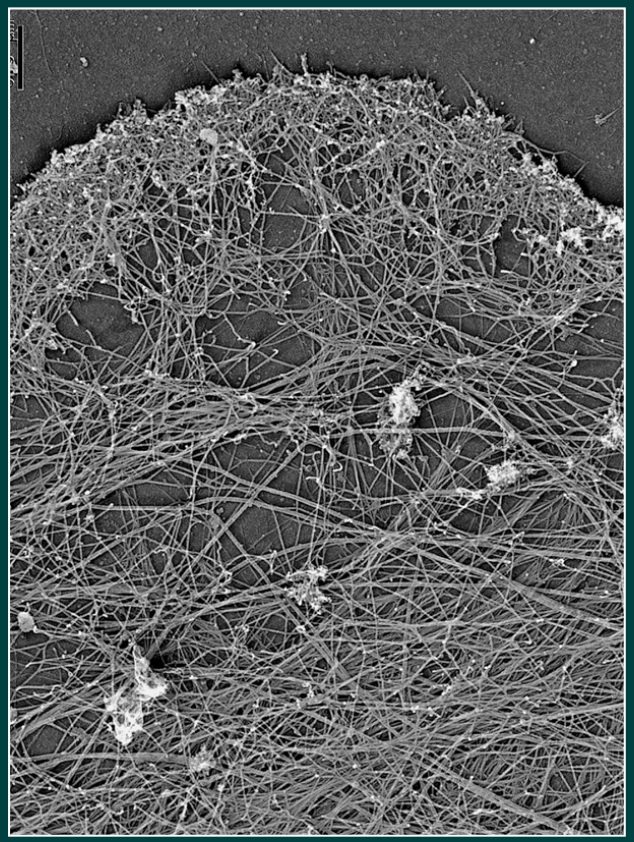
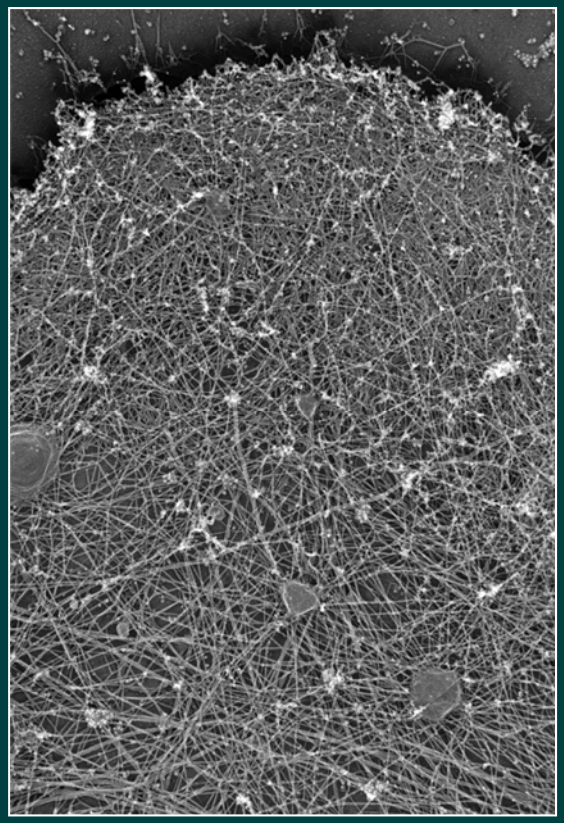
STRUCTURE OF LAMELLIPODIA AFTER MYOSIN II INHIBITION

Blebbistatin treatment

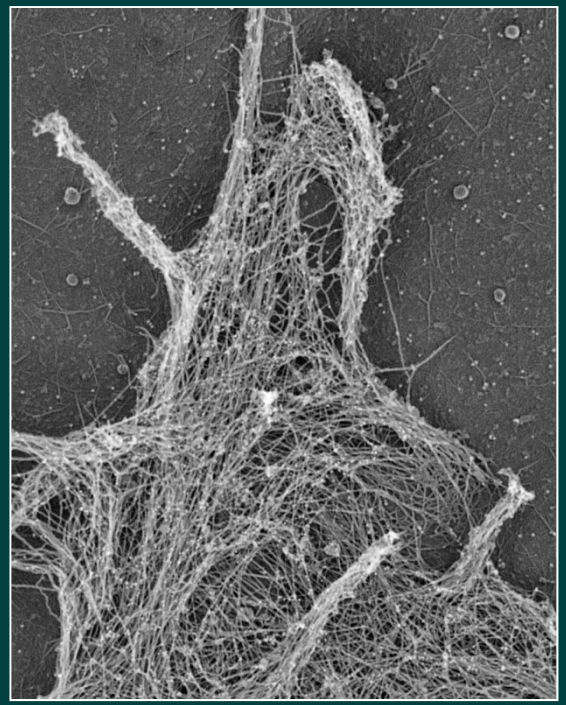
Control

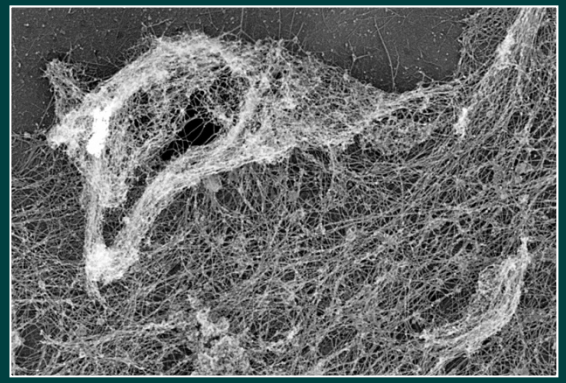


75 μM



100 μM





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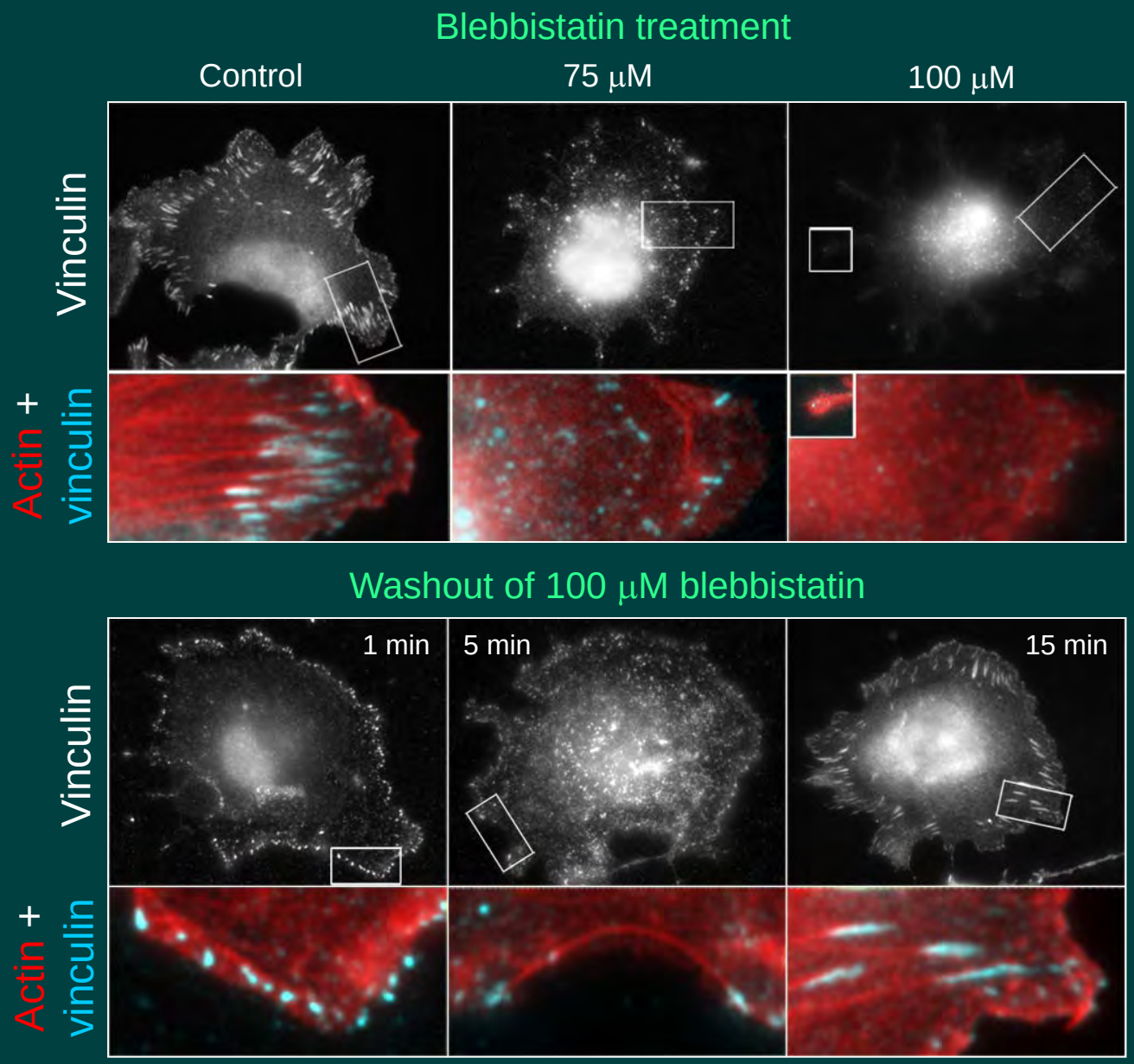
FOCAL COMPLEXES DEPEND ON MYOSIN II ACTIVITY



Myosin II motor activity is required for formation of lamellipodia and focal complexes

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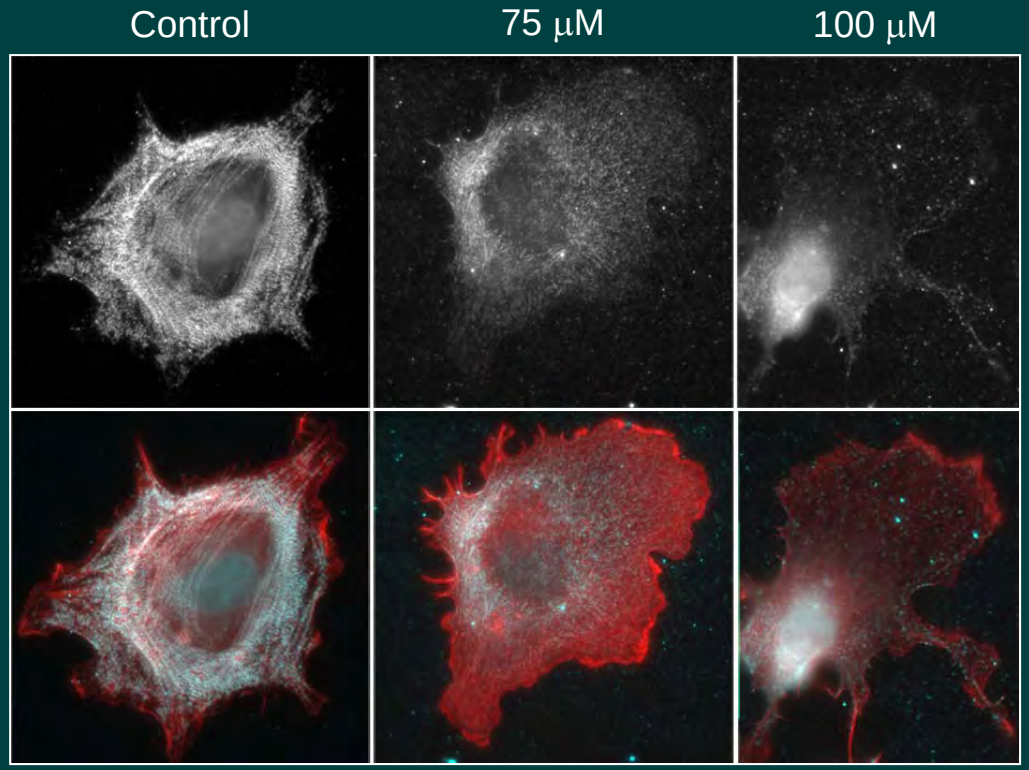
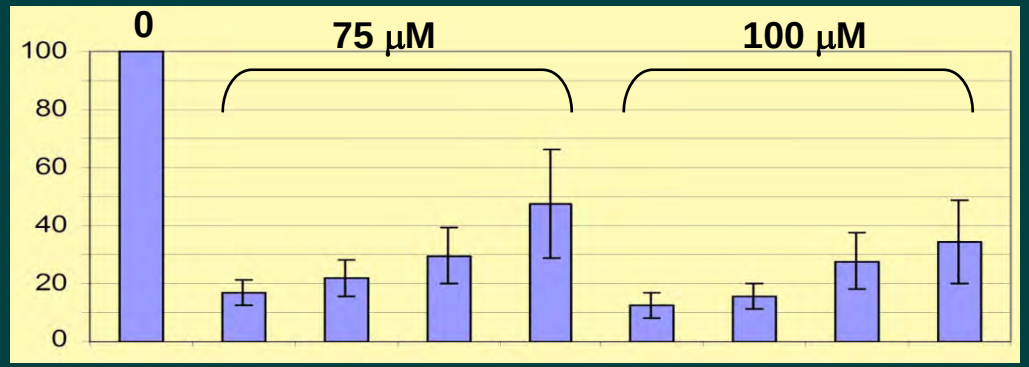
INACTIVE MYOSIN II DISSOCIATES FROM THE CYTOSKELETON

Blebbistatin treatment





Washout (min): 0 0 1 5 15 0 1 5 15

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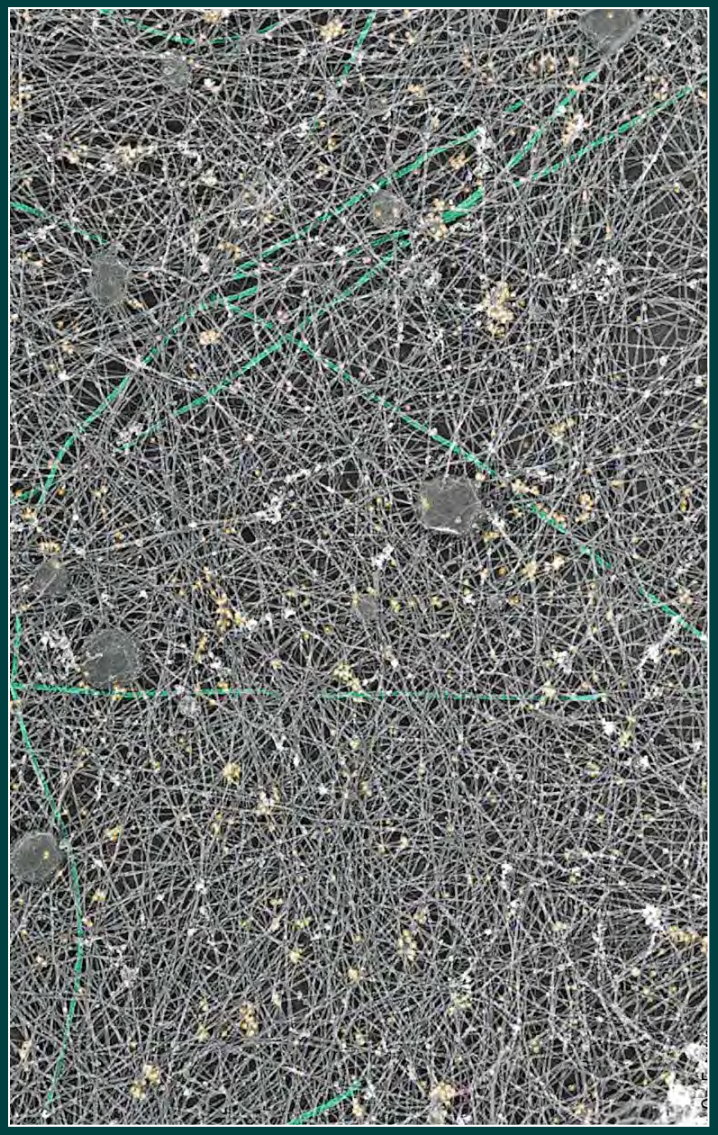
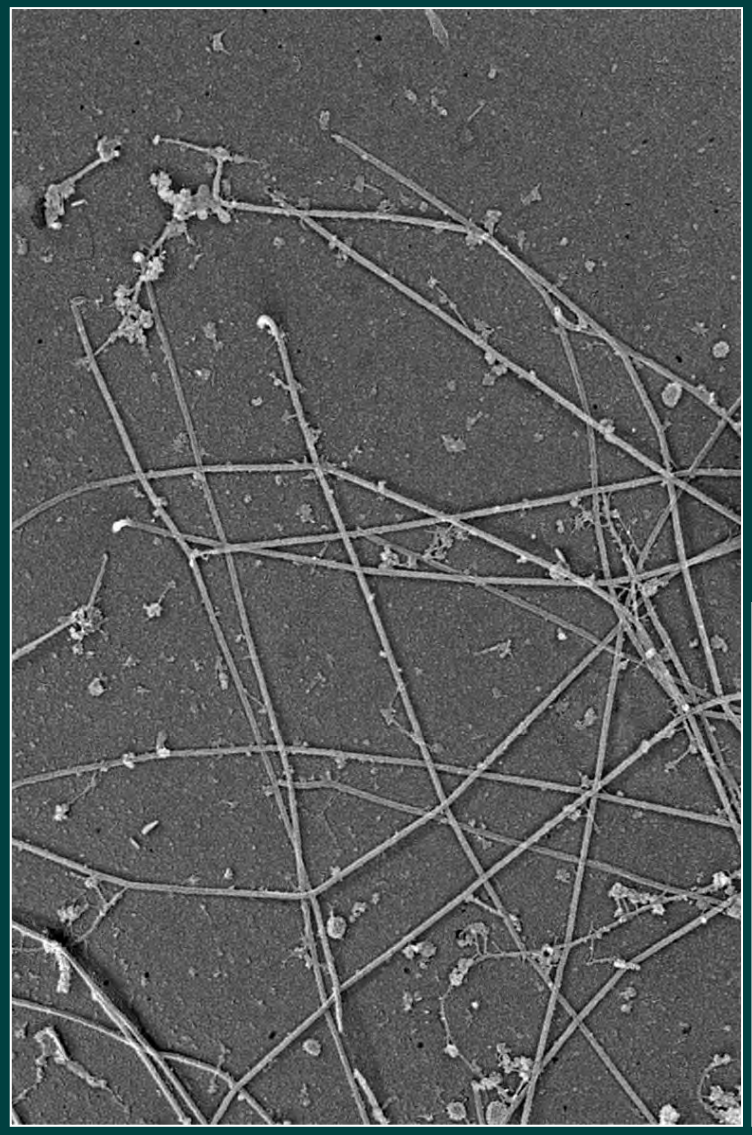
MYOSIN II FILAMENTS DISASSEMBLE AFTER BLEBBISTATIN TREATMENT (75 μM)



Microtubules

Myosin II immunogold



Gelsolin treatment

Myosin II immunogold

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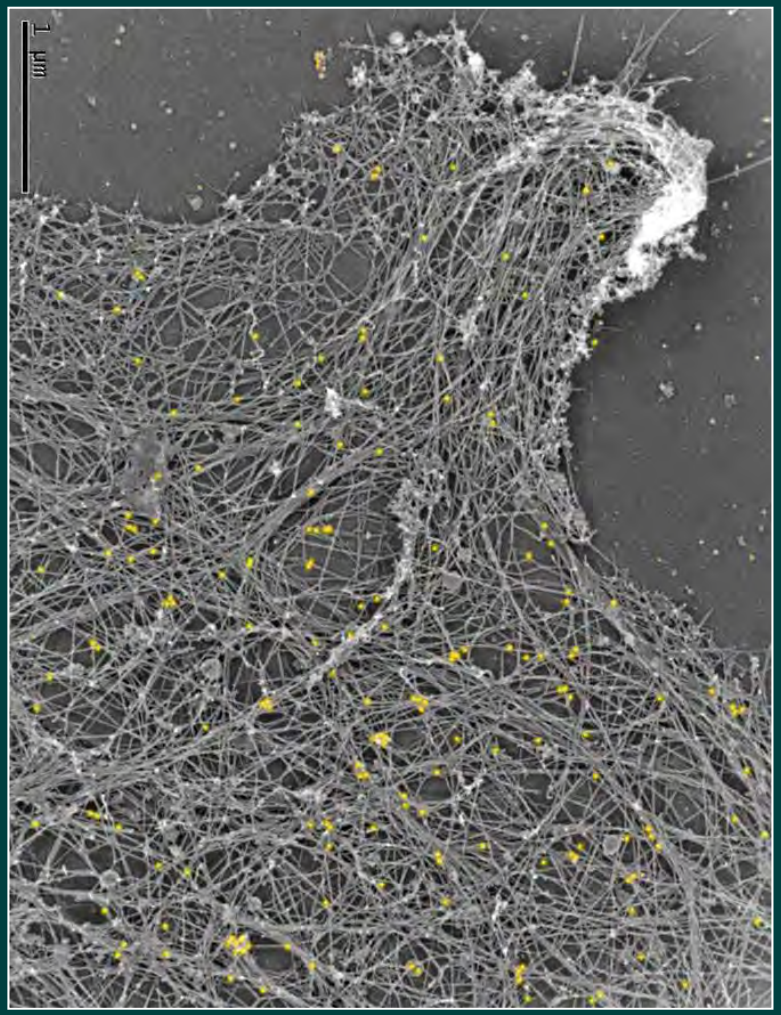
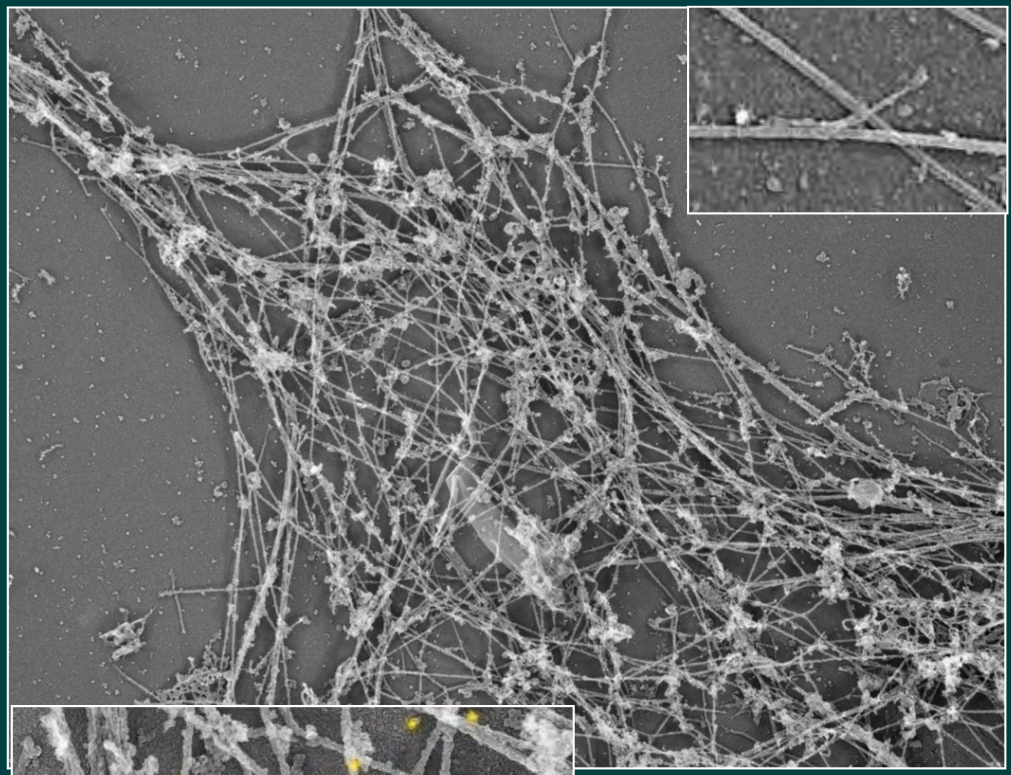
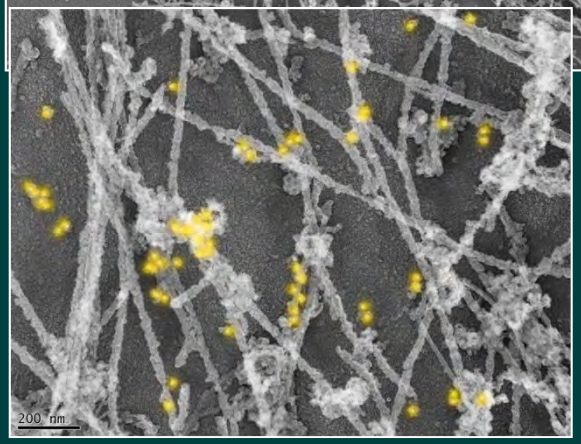
MYOSIN II FILAMENTS DISASSEMBLE AFTER BLEBBISTATIN TREATMENT (100 μM)

Myosin II - ImmunoGold



Gelsolin treatment





Gelsolin treatment +
Myosin II immunogold

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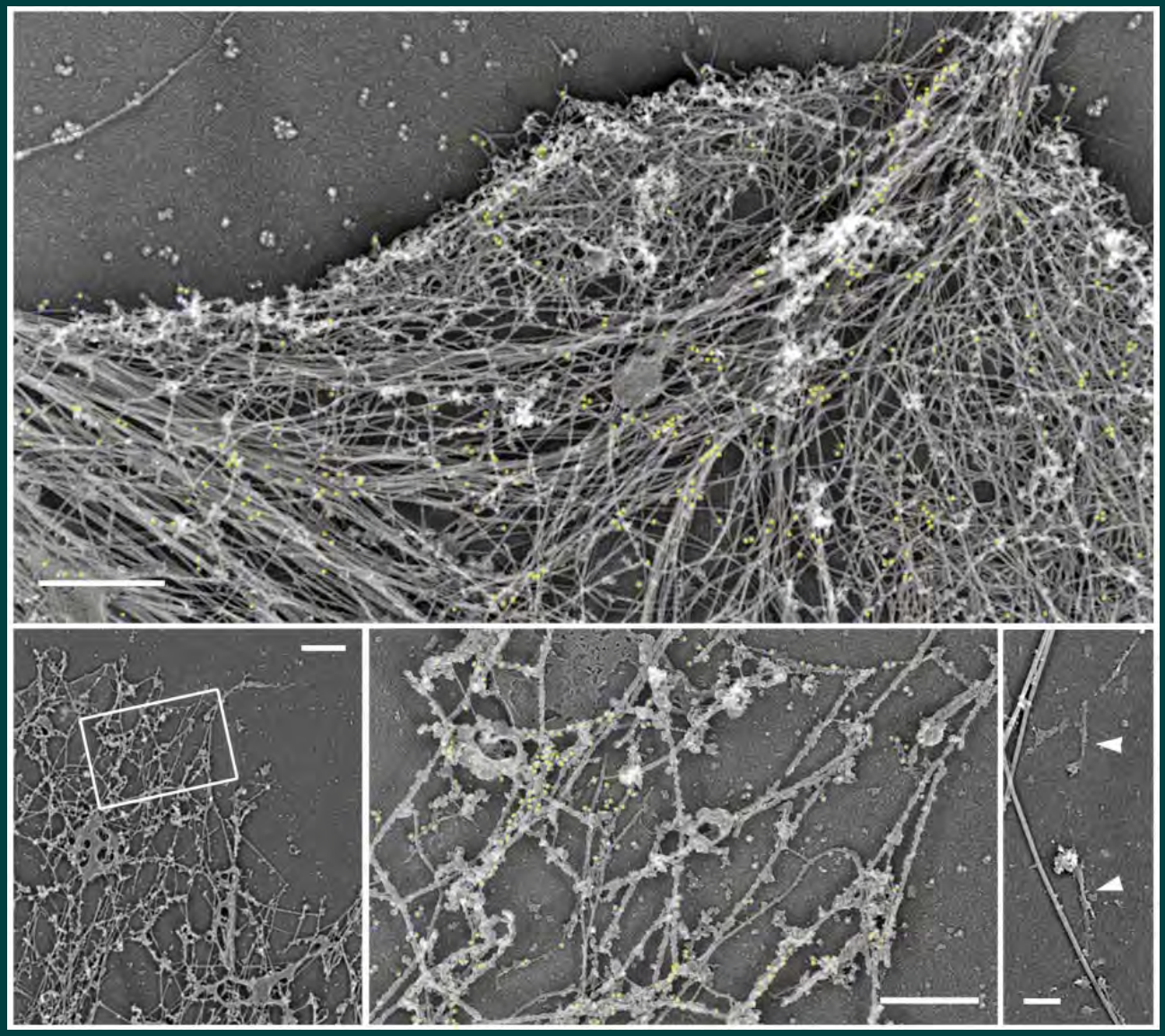
SLOW REFORMATION OF MYOSIN II FILAMENTS AFTER BLEBBISTATIN WASHOUT (1 MIN)



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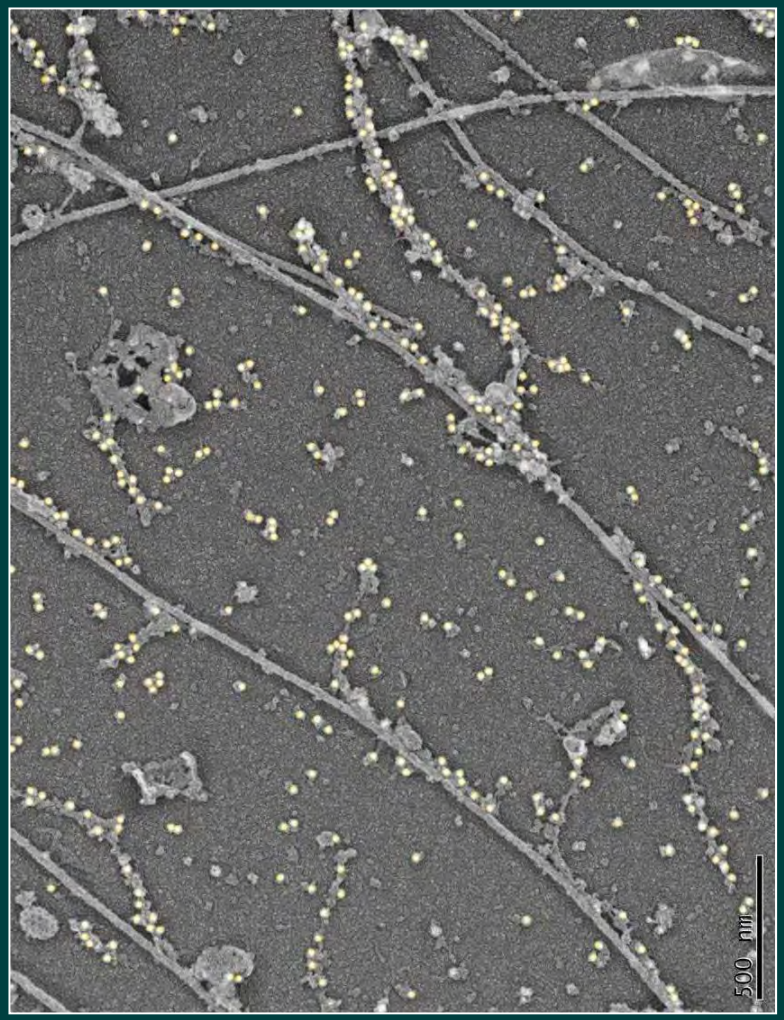
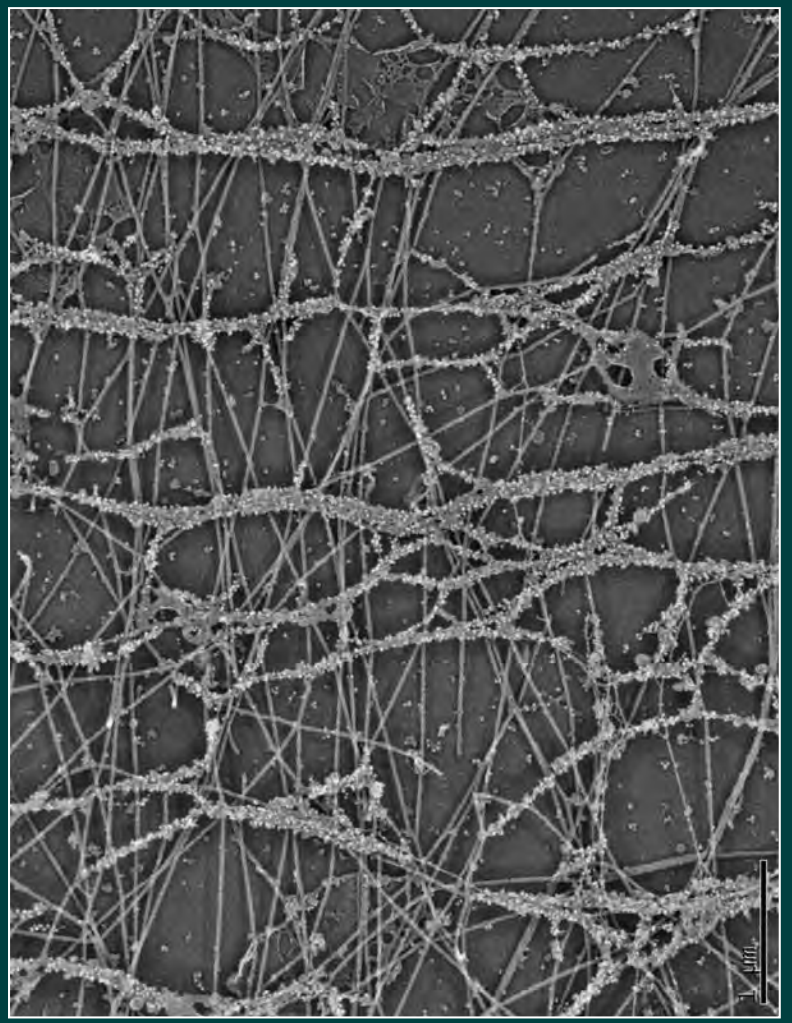
SLOW RECOVERY OF MYOSIN II FILAMENTS AFTER BLEBBISTATIN WASHOUT

Washout of 100 μM blebbistatin



5 min



15 min

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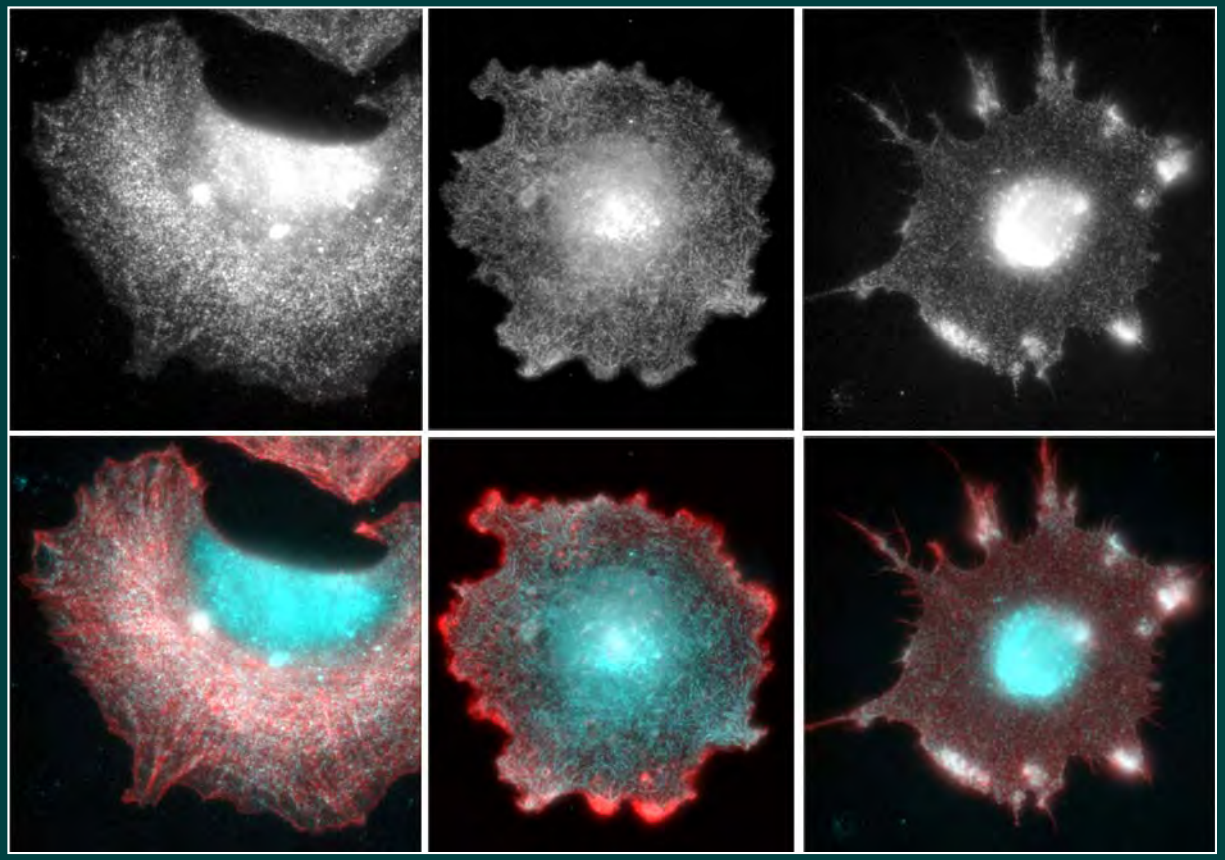
MYOSIN LIGHT CHAIN REMAINS PHOSPHORYLATED AFTER BLEBBISTATIN TREATMENT

Blebbistatin treatment

Control | 75 μM | 100 μM



When myosin motor activity is inhibited, myosin II bipolar filaments disassemble, despite persisting MRLC phosphorylation

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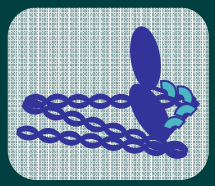
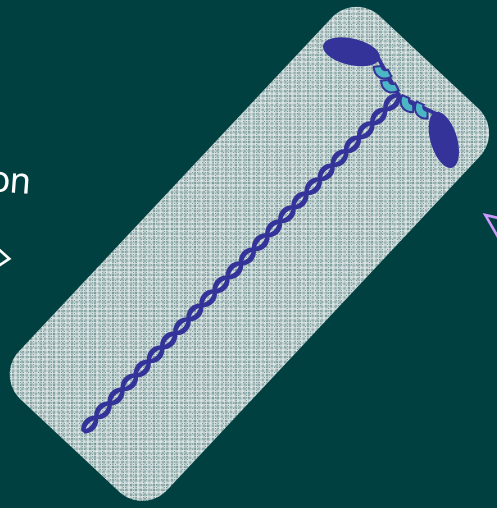
MYOSIN II POLYMERIZATION MAY BE REGULATED IN TENSION-DEPENDENT MANNER

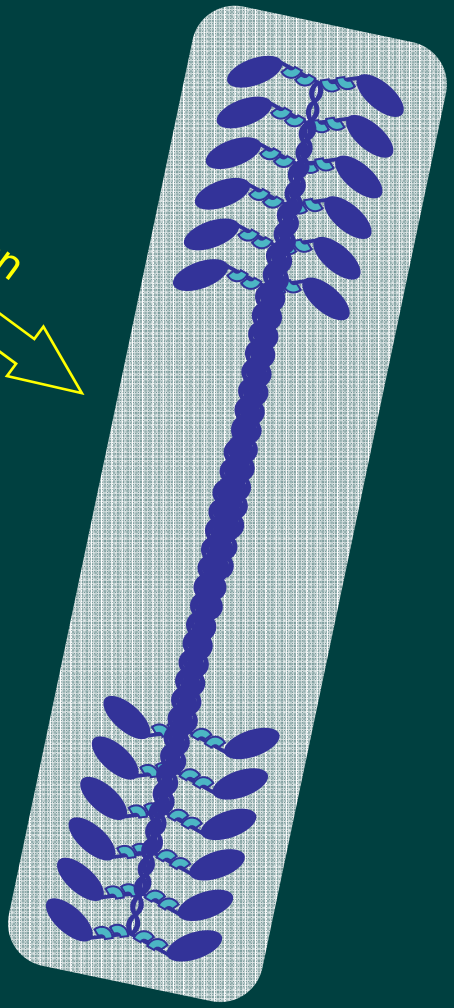


MRLC phosphorylation



Tension

Relaxation



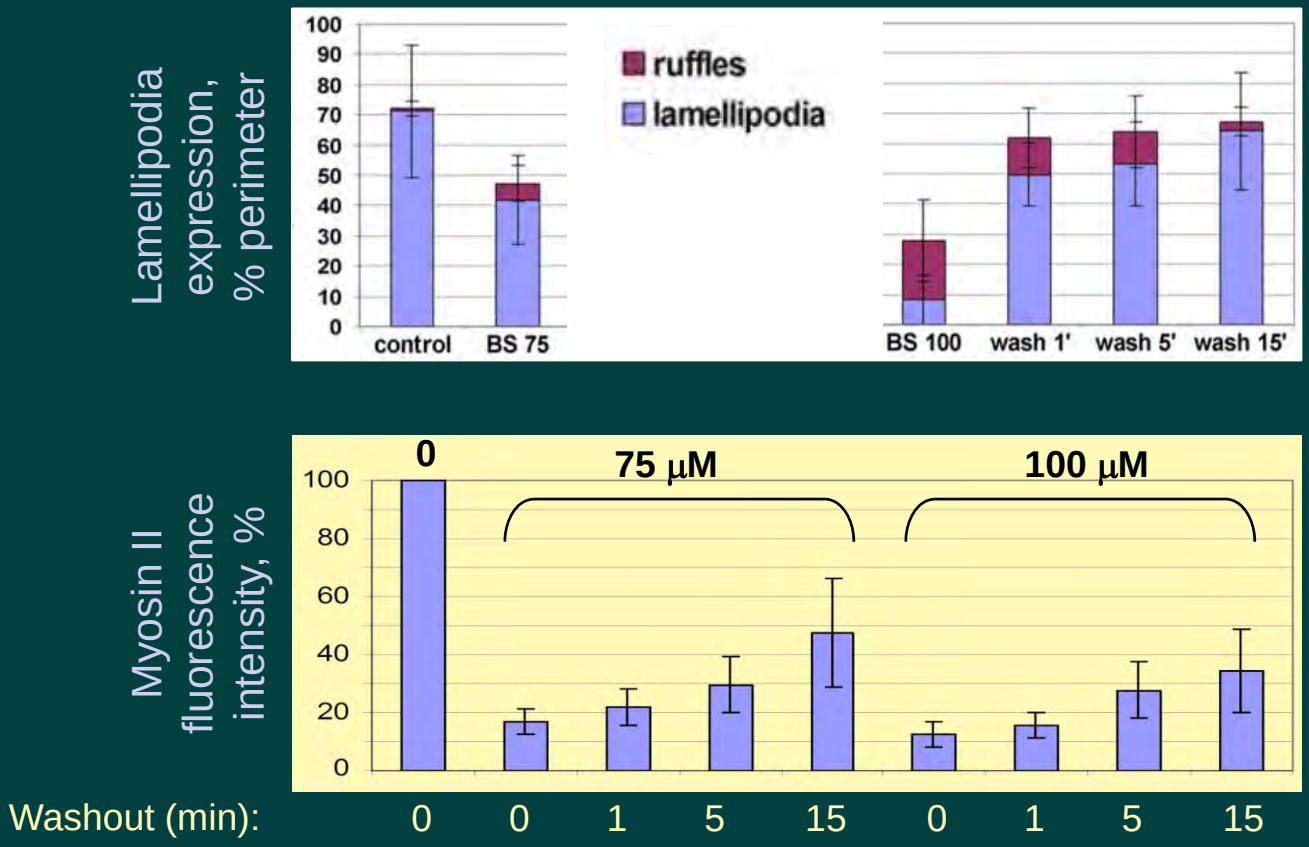
Ren et al, 2009

Myosin II

Phosphorylation, S100A4 (Mts1)

MHC (polymerization)

LAMELLIPODIA RECOVER SOONER THAN THE CYTOSKELETAL ASSOCIATION OF MYOSIN II



Myosin II promotes formation of lamellipodia and focal complexes before assembling into bipolar filaments

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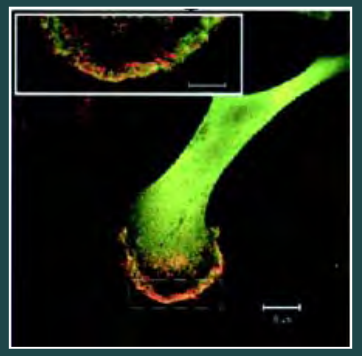
MYOSIN II POLYMERIZATION MAY BE NEGATIVELY REGULATED IN PROTRUSIONS

Phosphorylation,
S100A4 (Mts1)

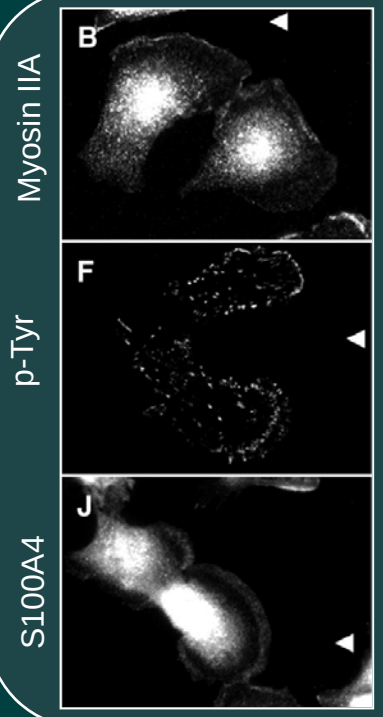
MHC (polymerization)



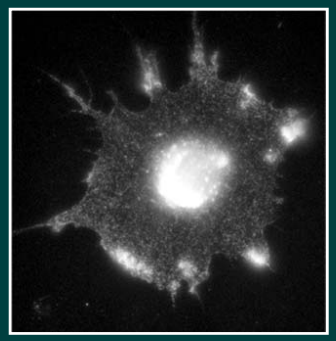
Arp3
S100A4

Kim and Helfman, 2003

Rosenberg and Ravid, 2006

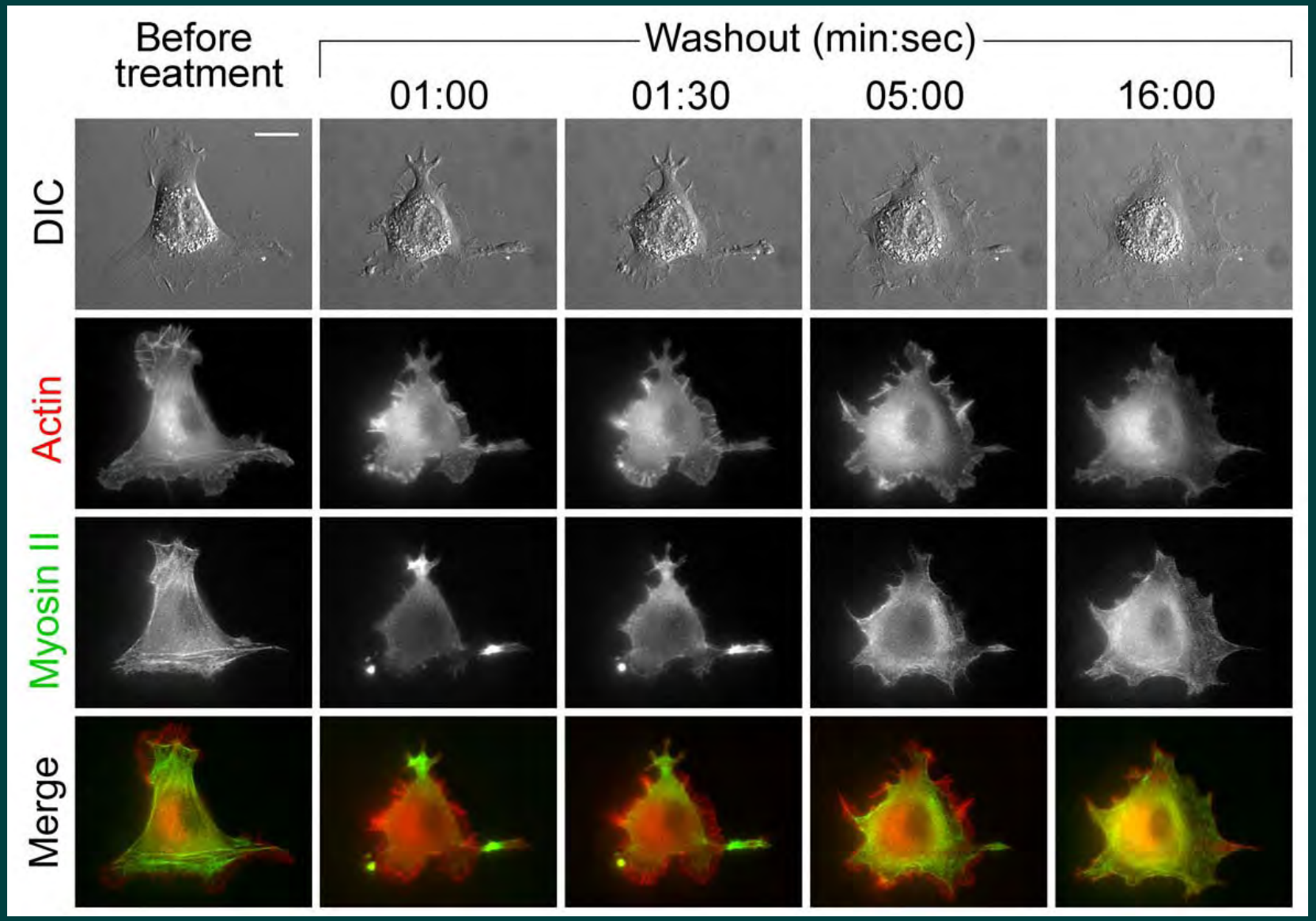


Li and Bresnick, 2006



Myosin II is double phosphorylated in protrusions, but its polymerization there is inhibited

MYOSIN II MOVES CENTRIPETALLY AFTER BLEBBISTATIN WASHOUT



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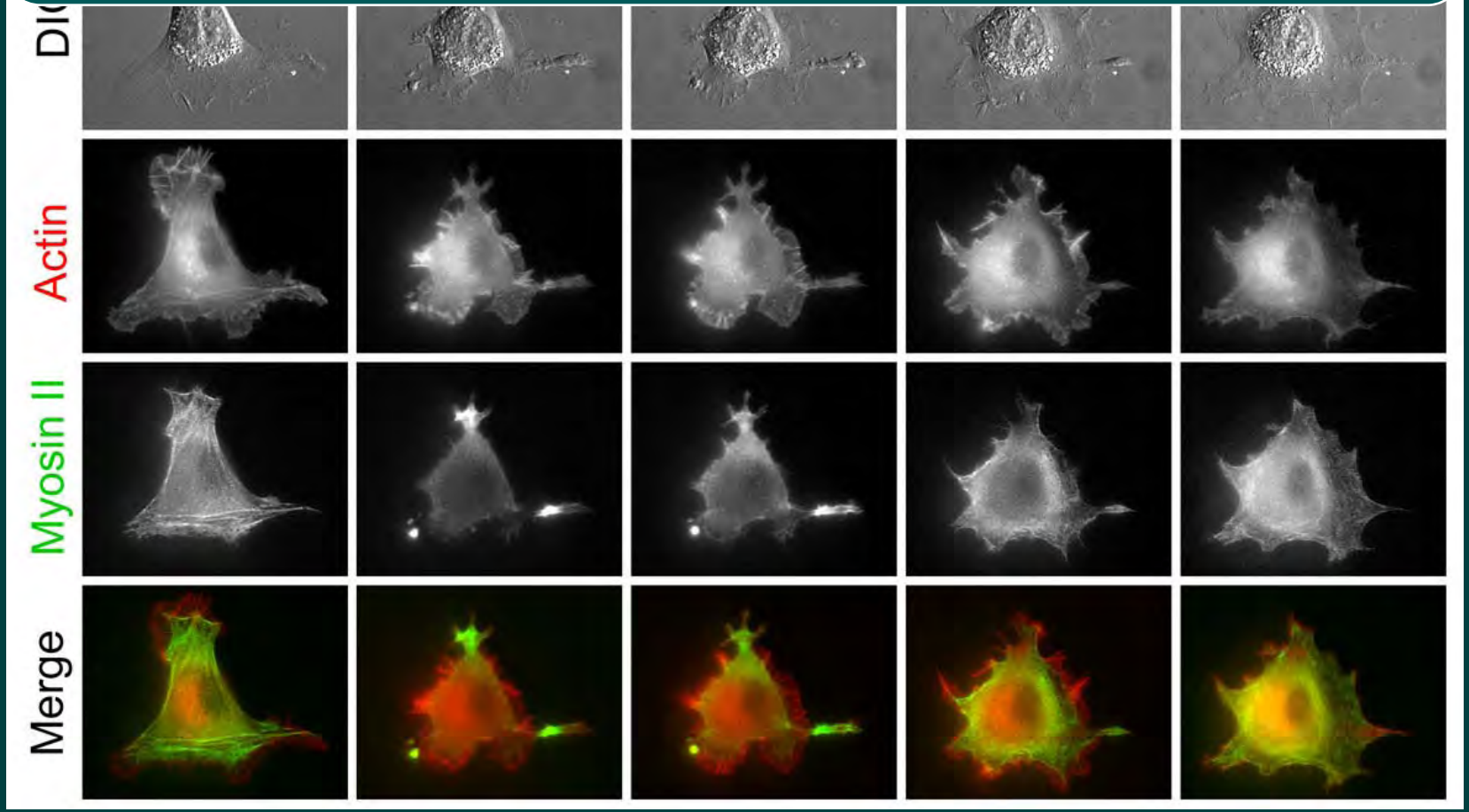
MYOSIN II MOVES CENTRIPETALLY AFTER BLEBBISTATIN WASHOUT

Activated unpolymerized myosin II quickly leaves protrusions in motor activity-dependent manner



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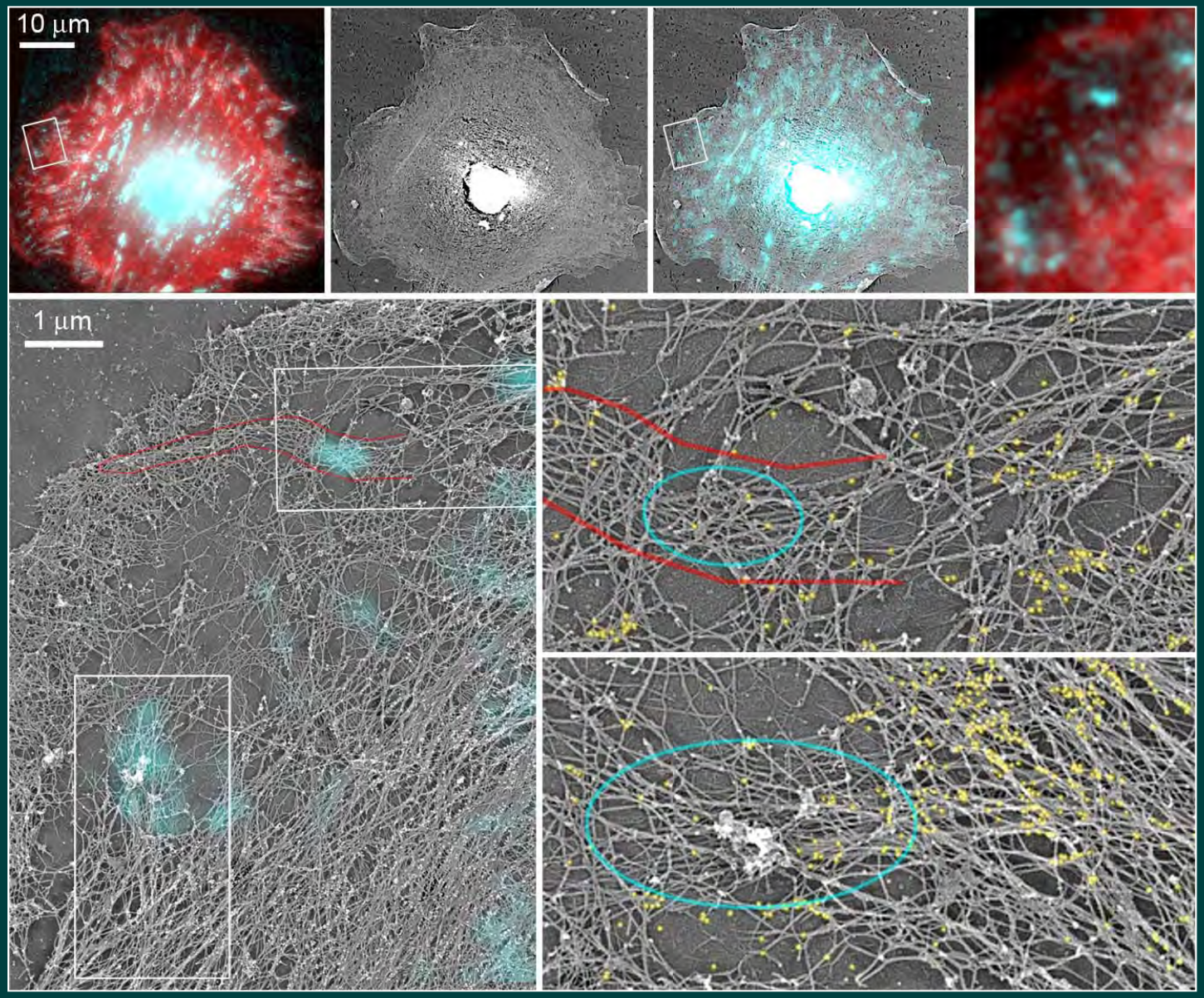
VISUALIZATION OF KEY COMPONENTS OF THE CONTRACTILE SYSTEM



F-actin

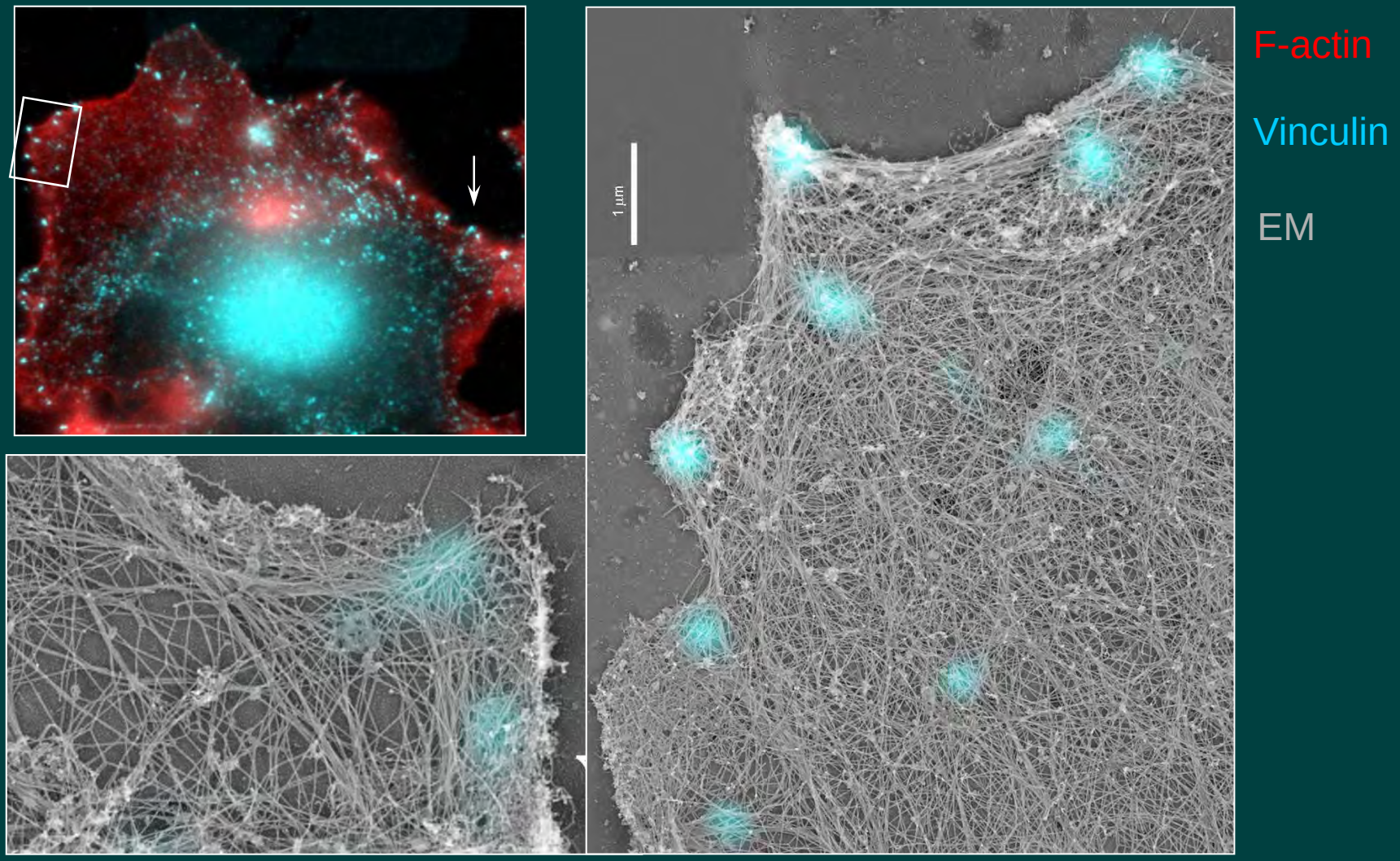
Vinculin

Myosin II

EM

Maria Shutova

FOCAL COMPLEXES ARE INITIALLY FORMED UNDER FILOPODIA AND CONCAVE ACTIN ARCS



Blebbistatin washout (1 min)

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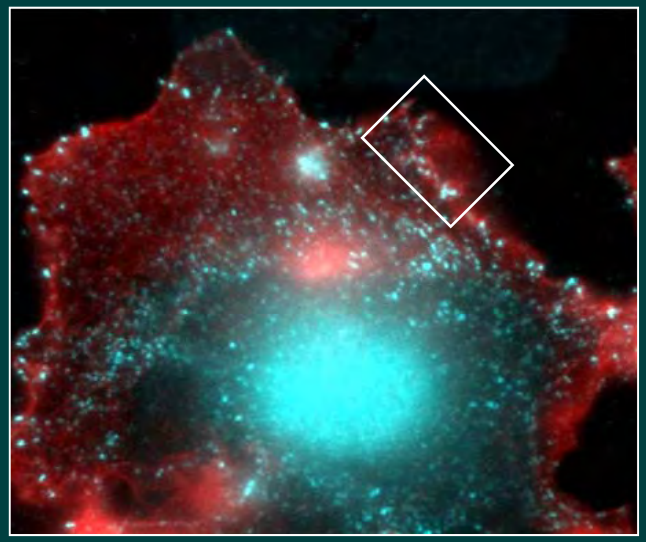
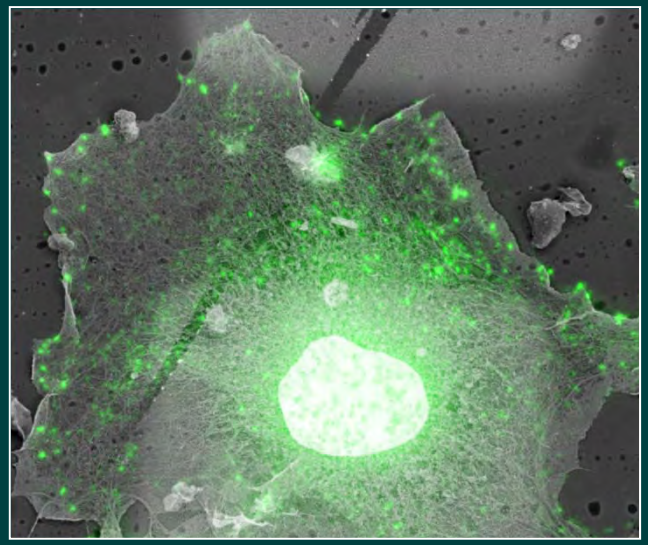
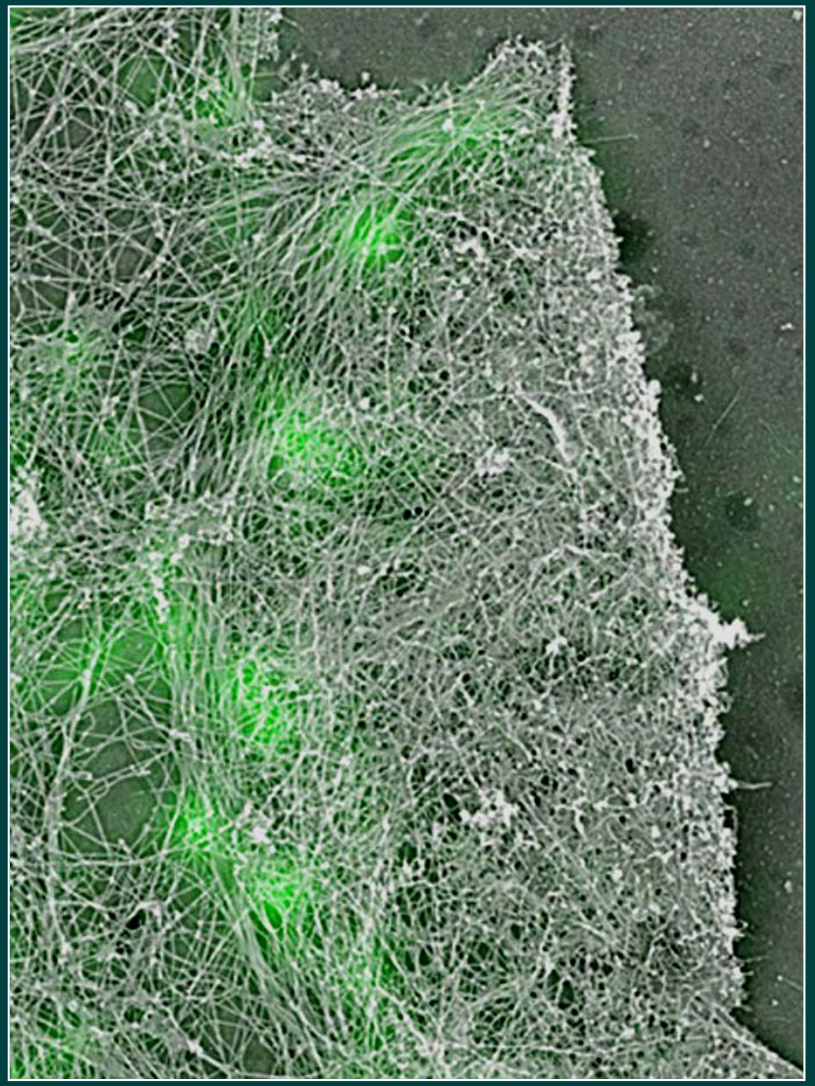
FOCAL COMPLEXES ARE INITIALLY FORMED UNDER FILOPODIA AND CONCAVE ACTIN ARCS







F-actin

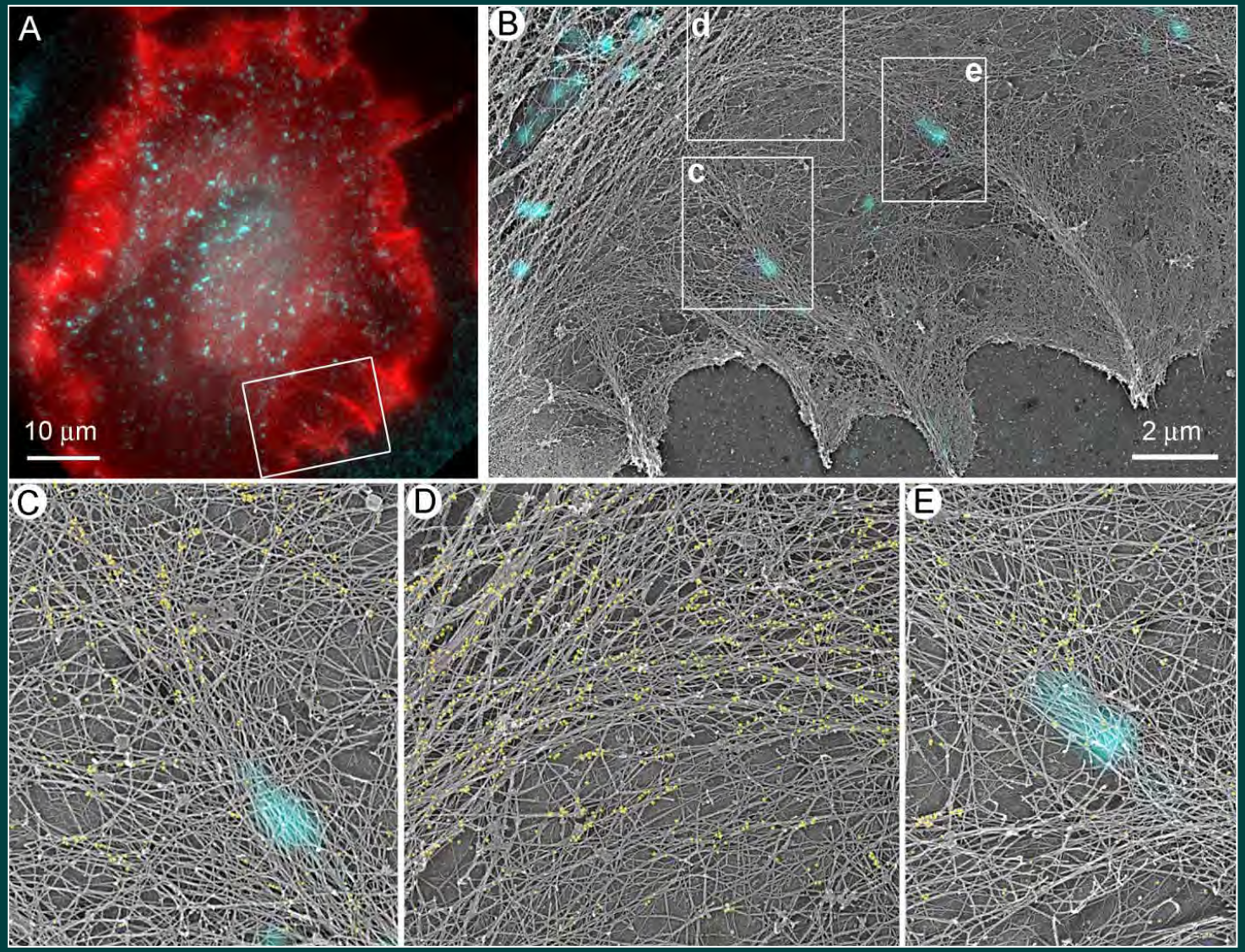
Vinculin

EM

Blebbistatin washout (1 min)

Maria Shutova

MYOSIN II BEGINS TO ACCUMULATE IN FILOPODIAL ROOTS AND CONCAVE ARCS



F-actin

Vinculin

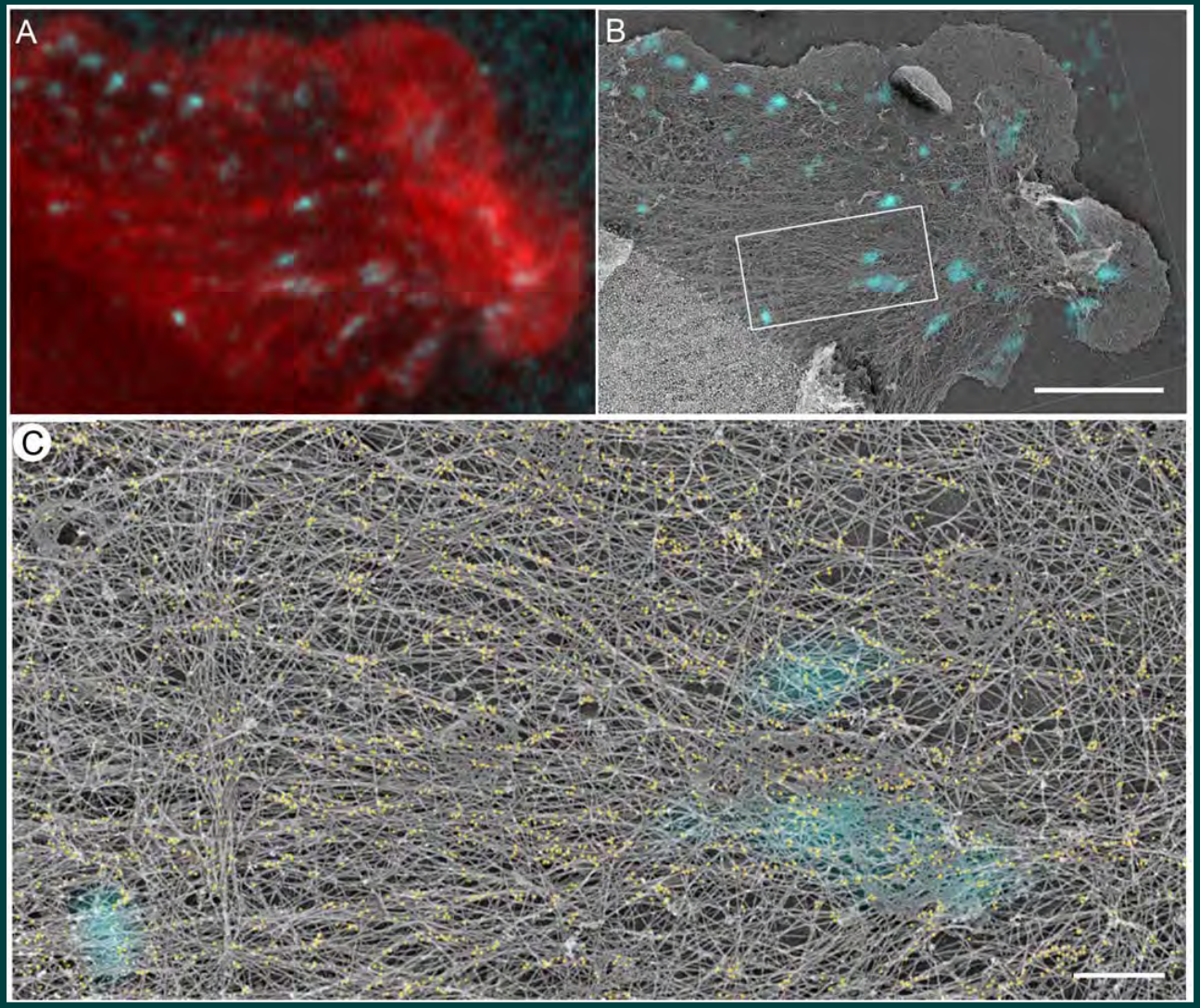
Myosin II

EM

Blebbistatin washout (5 min)

Maria Shutova

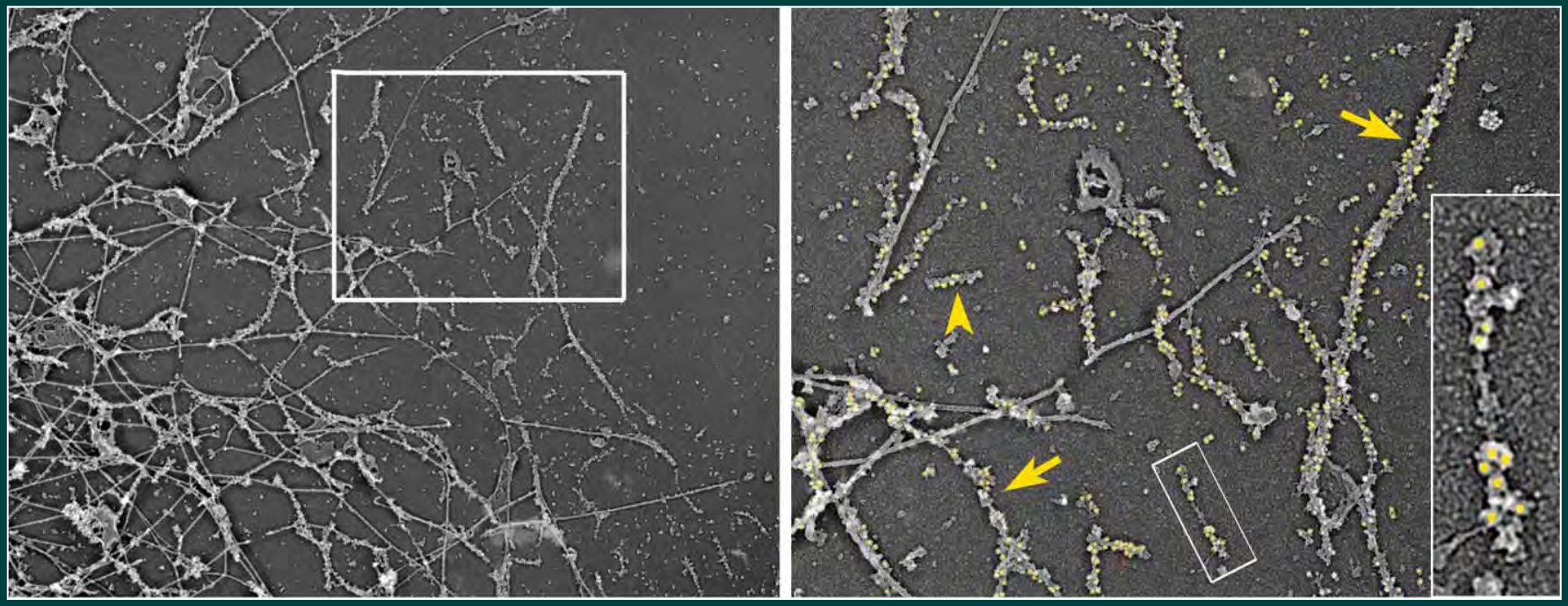
ORGANIZATION OF ACTIN, MYOSIN II AND FOCAL ADHESION IN NASCENT STRESS FIBERS



Blebbistatin washout (15 min)

Maria Shutova

MYOSIN II FILAMENTS APPEAR IN LAMELLA AND FORM CHAINS AT CONCAVE CELL EDGES



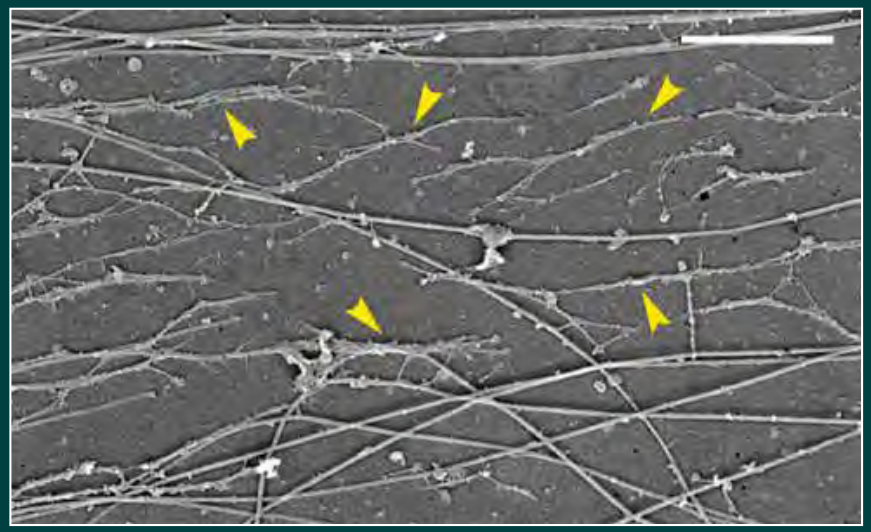
Blebbistatin washout (5 min)

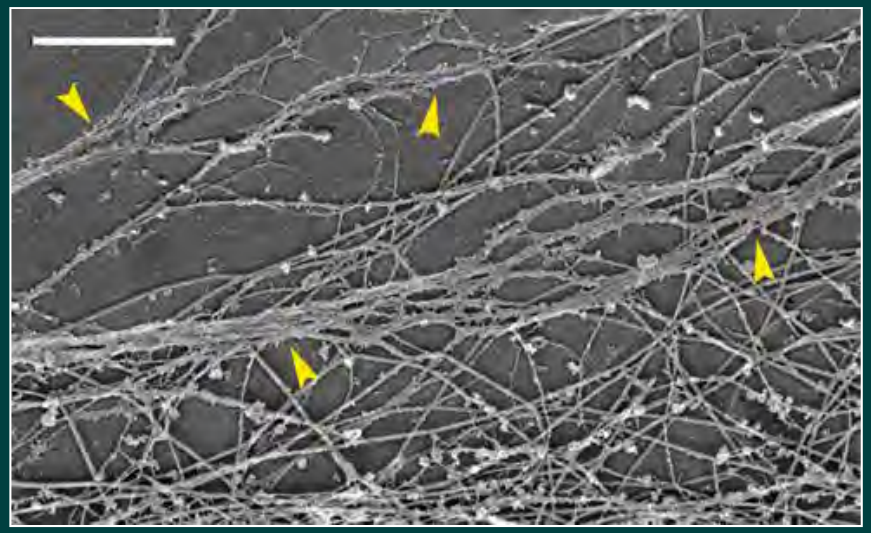
Maria Shutova

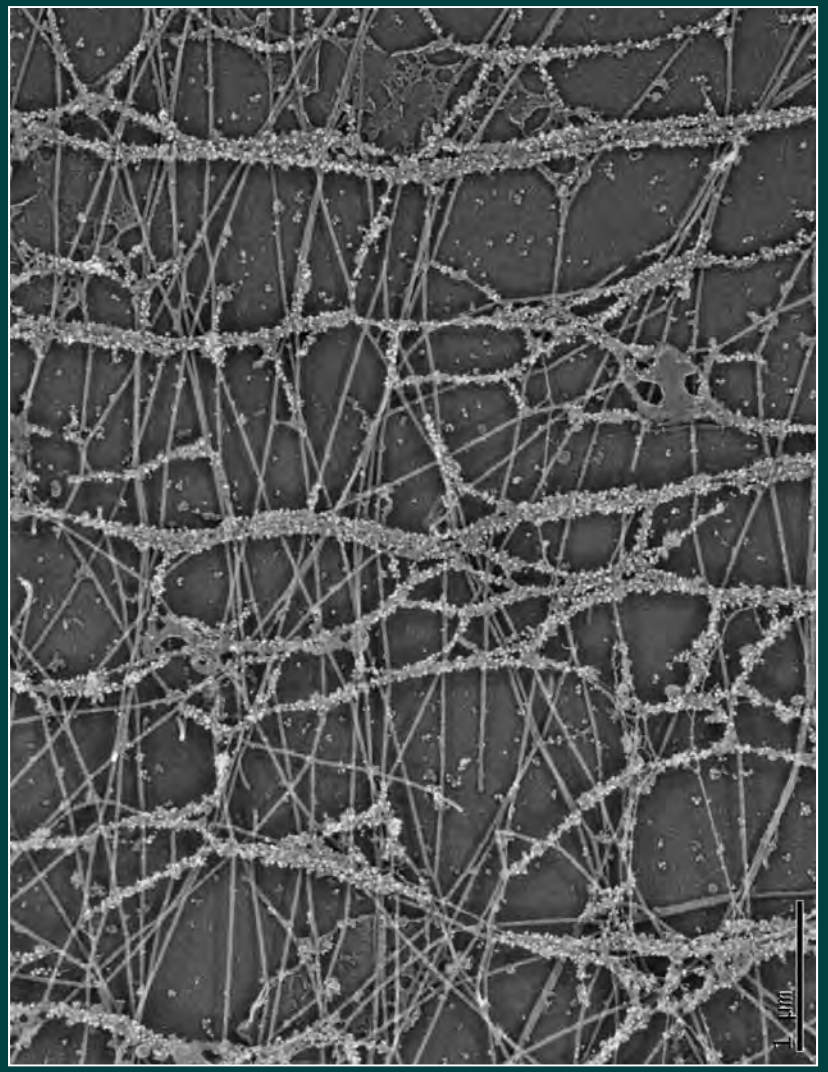
MYOSIN II FORMS CHAINS, BUT NOT STACKS, IN NASCENT STRESS FIBERS

Distal region

Proximal region







Maria Shutova

ROLES OF MYOSIN II IN ASSEMBLY OF THE CONTRACTILE SYSTEM

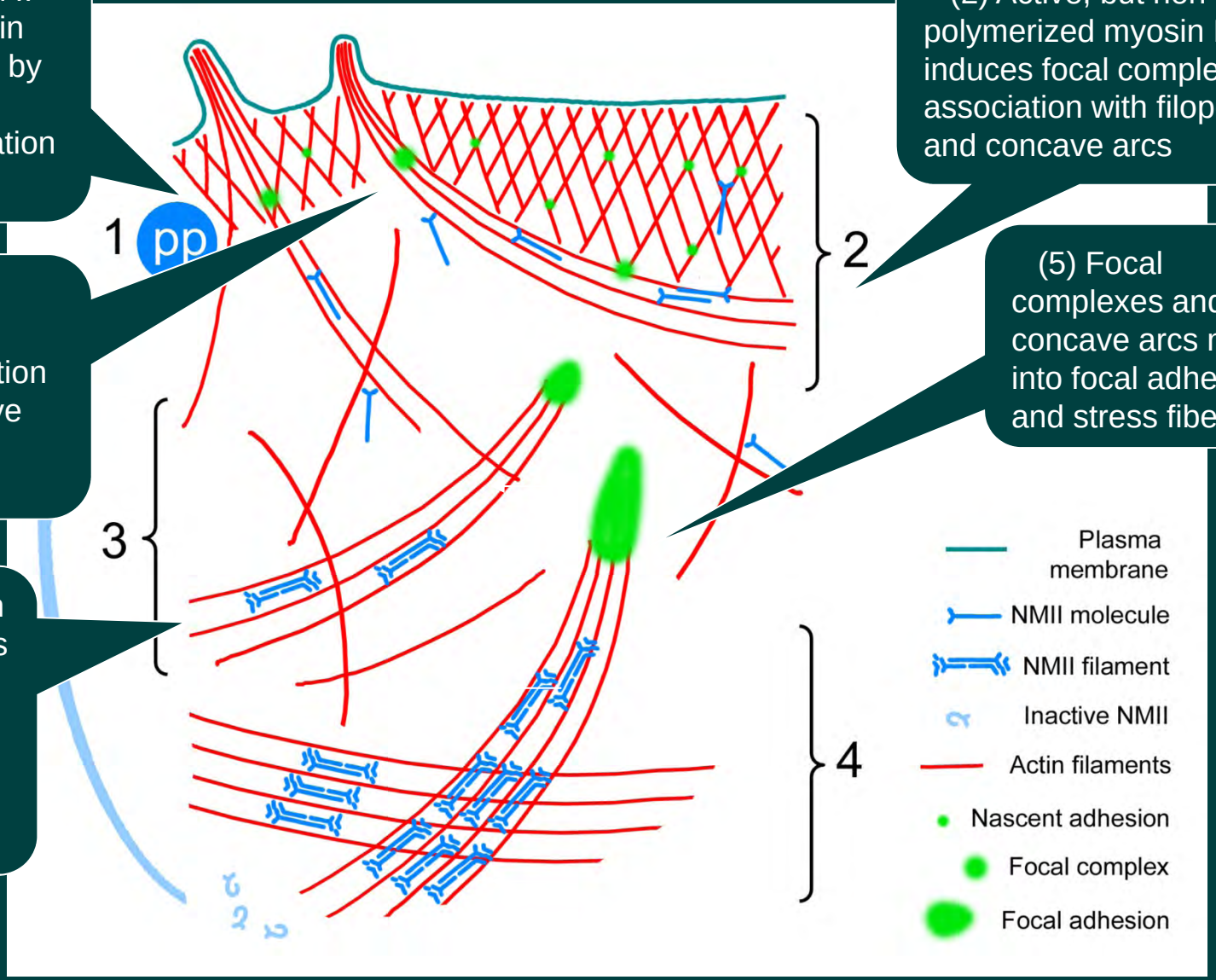
(1) Myosin II is activated in lamellipodia by double phosphorylation of MRLC.

(2) Active, but non-polymerized myosin II induces focal complexes in association with filopodia and concave arcs

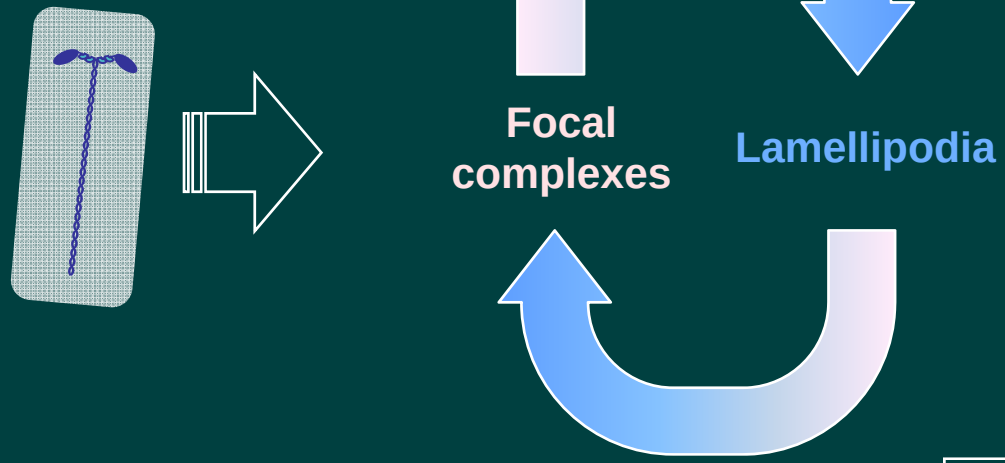
(3) Focal complexes provide traction for productive lamellipodia protrusion

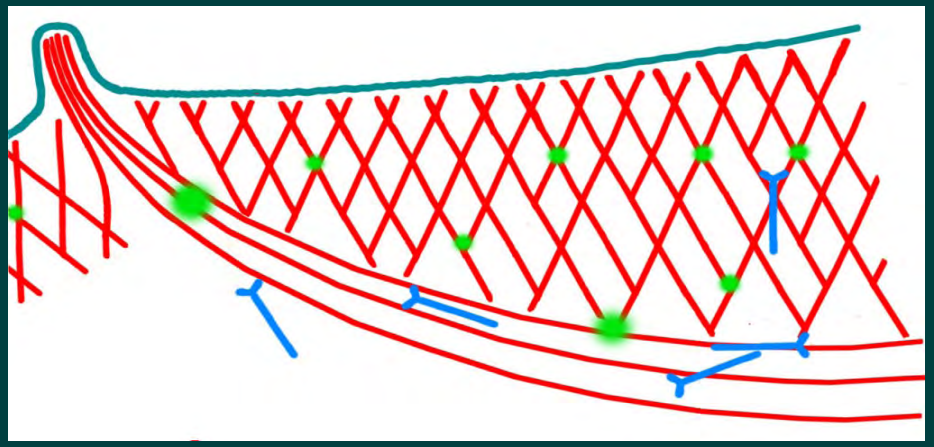
(5) Focal complexes and concave arcs mature into focal adhesions and stress fibers.

(4) Myosin II assembles into bipolar filaments in a load-dependent manner

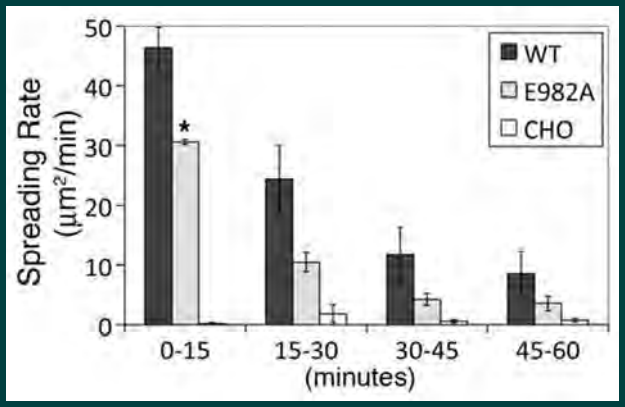


MUTUAL DEPENDENCE OF LAMELLIPODIA AND FOCAL COMPLEXES





Effect of disrupting α4–MIIA association on cell adhesion and spreading.



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