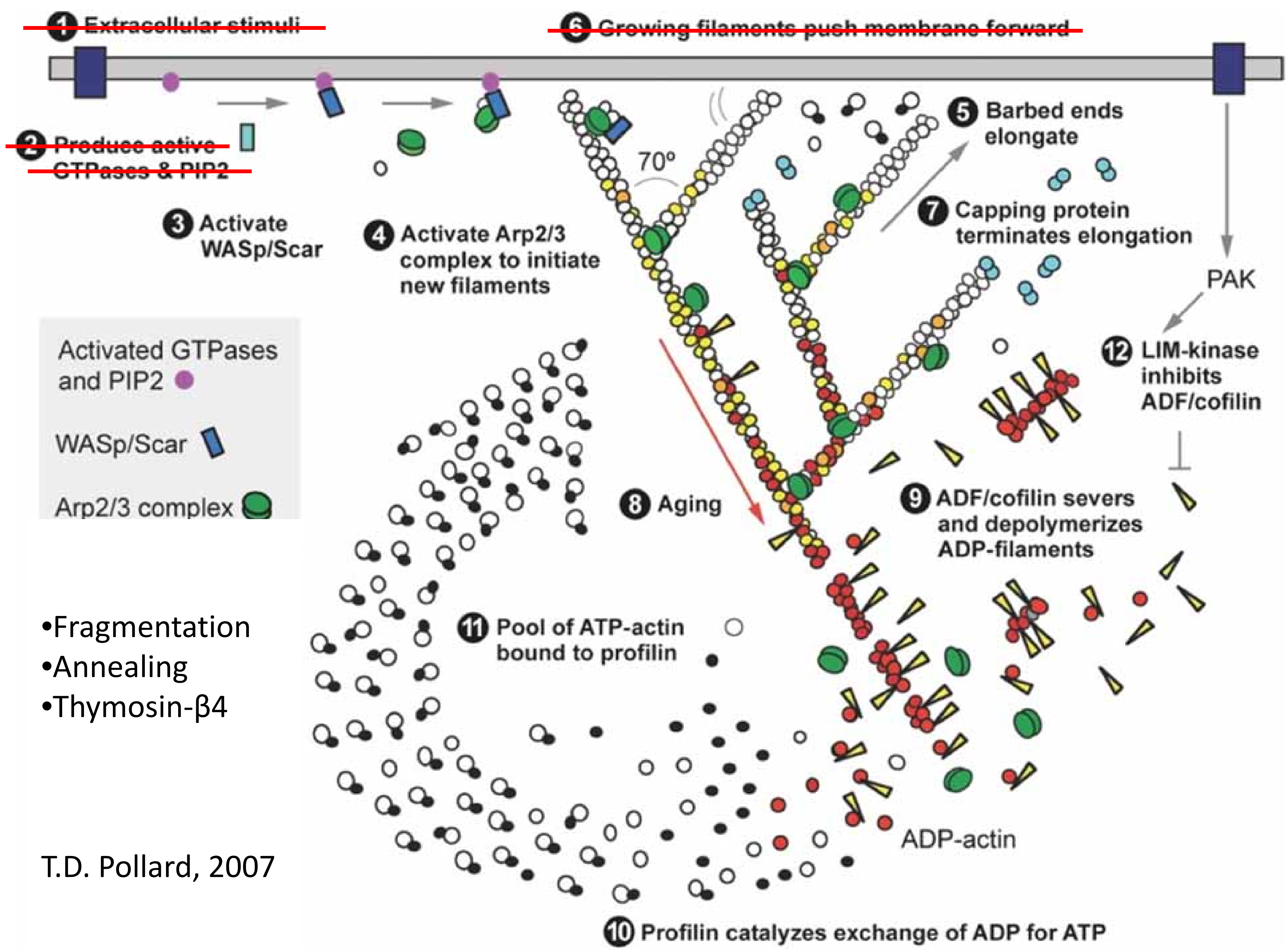
Modeling Actin Dynamics: Why the Details Matter

Jon Ditlev
Nate Vacanti
Igor Novak
Paul Michalski
Sofya Borinskaya

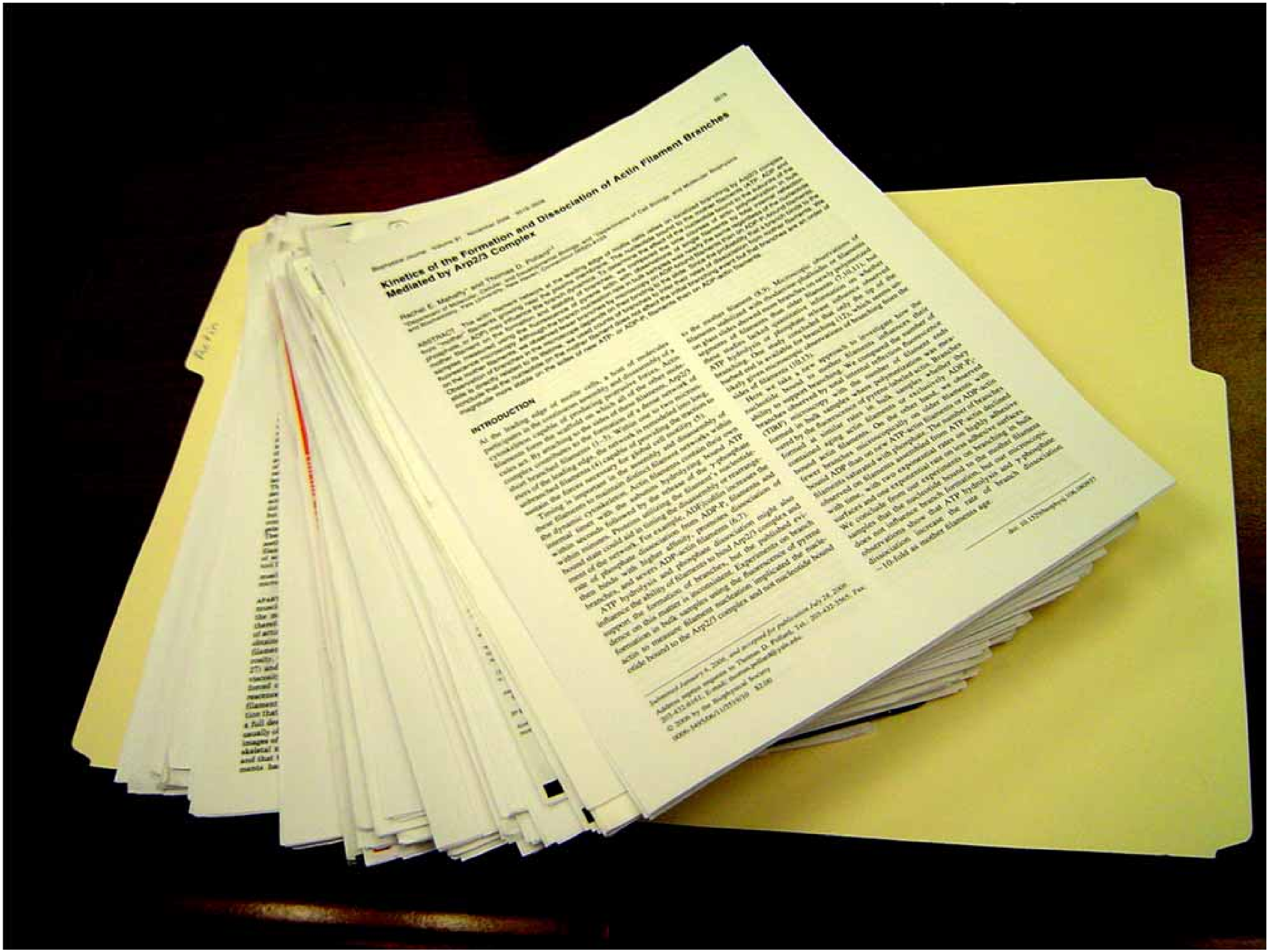
Collaborators: Maryna Kapustina, Ken Jacobson, Bruce Mayer
Advice: Boris Slepchenko, Pavel Kraikivsky, Jim Schaff, Tom Pollard

Modeling Actin Dynamics: Why the Details Matter



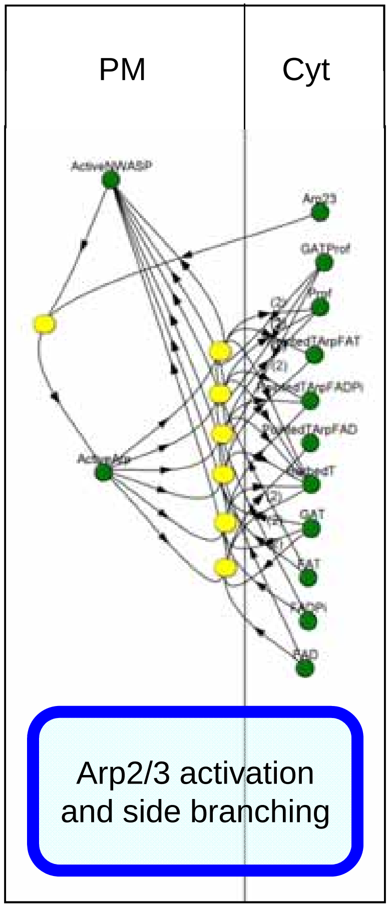
- Fragmentation
- Annealing
- Thymosin-β4

T.D. Pollard, 2007

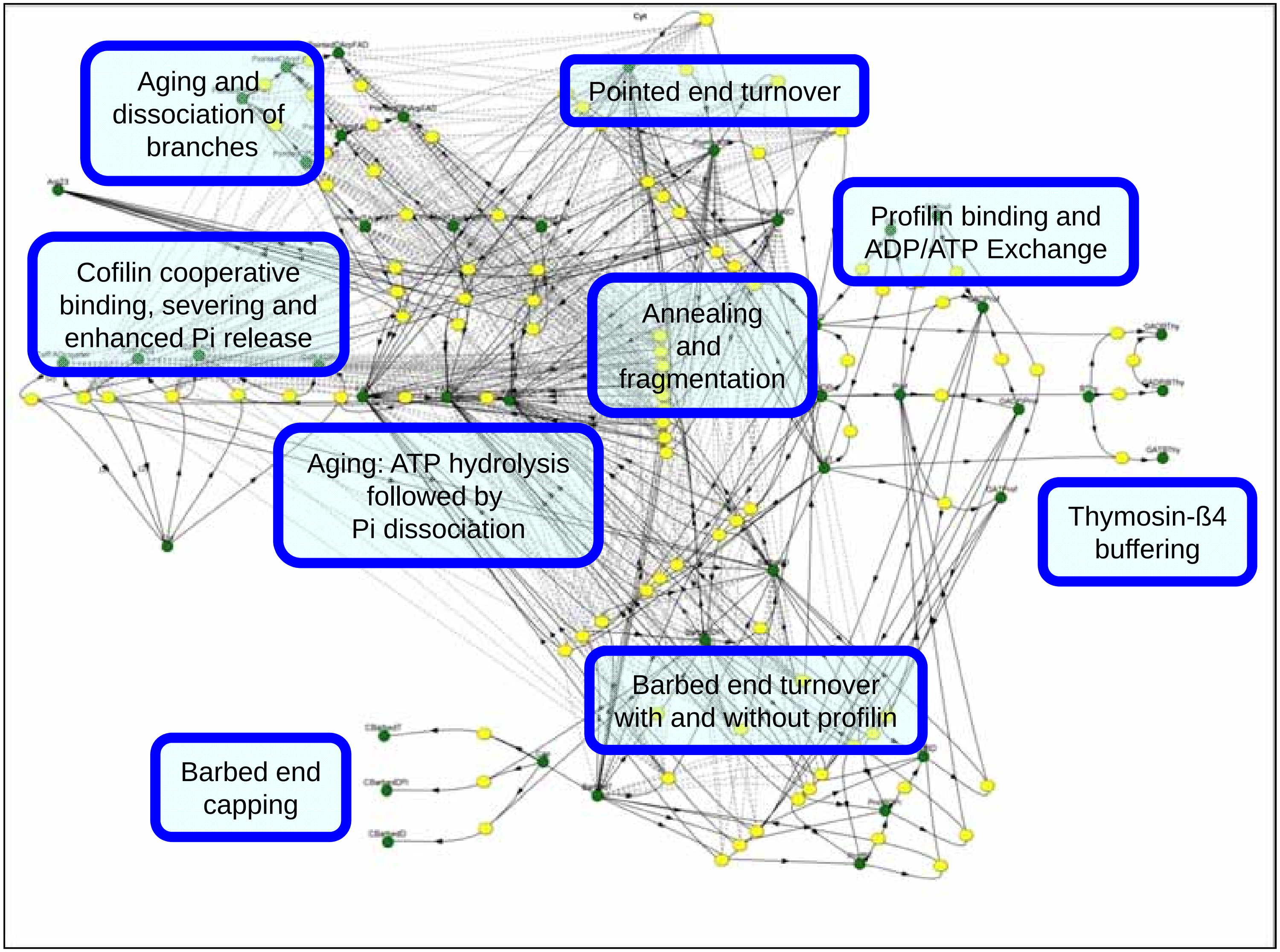

Kinetics of the Formation and Dissociation of Actin Filament Branches Mediated by Arp2/3 Complex
INTRODUCTION

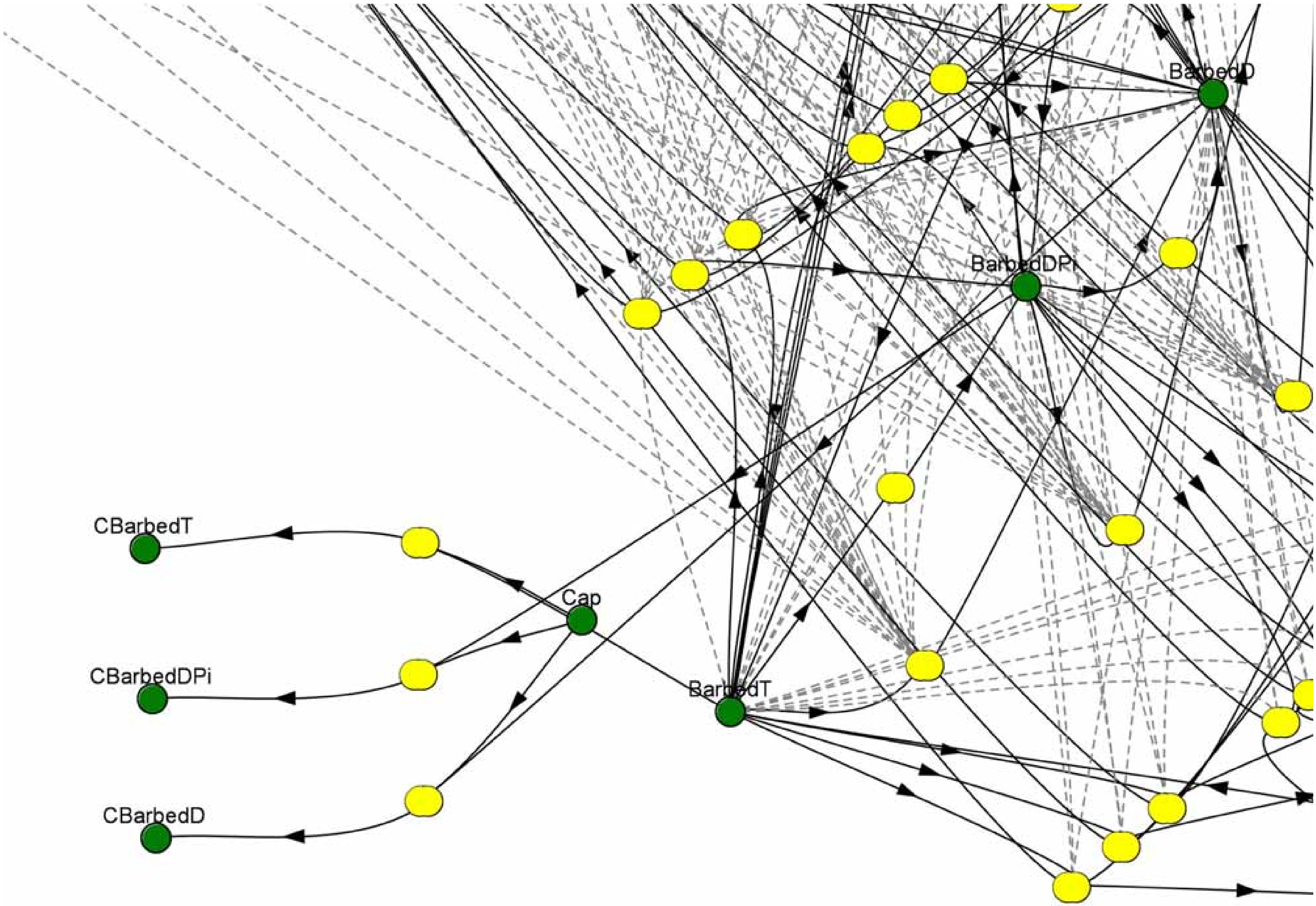
An Open Model of Actin Dendritic Nucleation
Biophys. J., 2009

At the Membrane



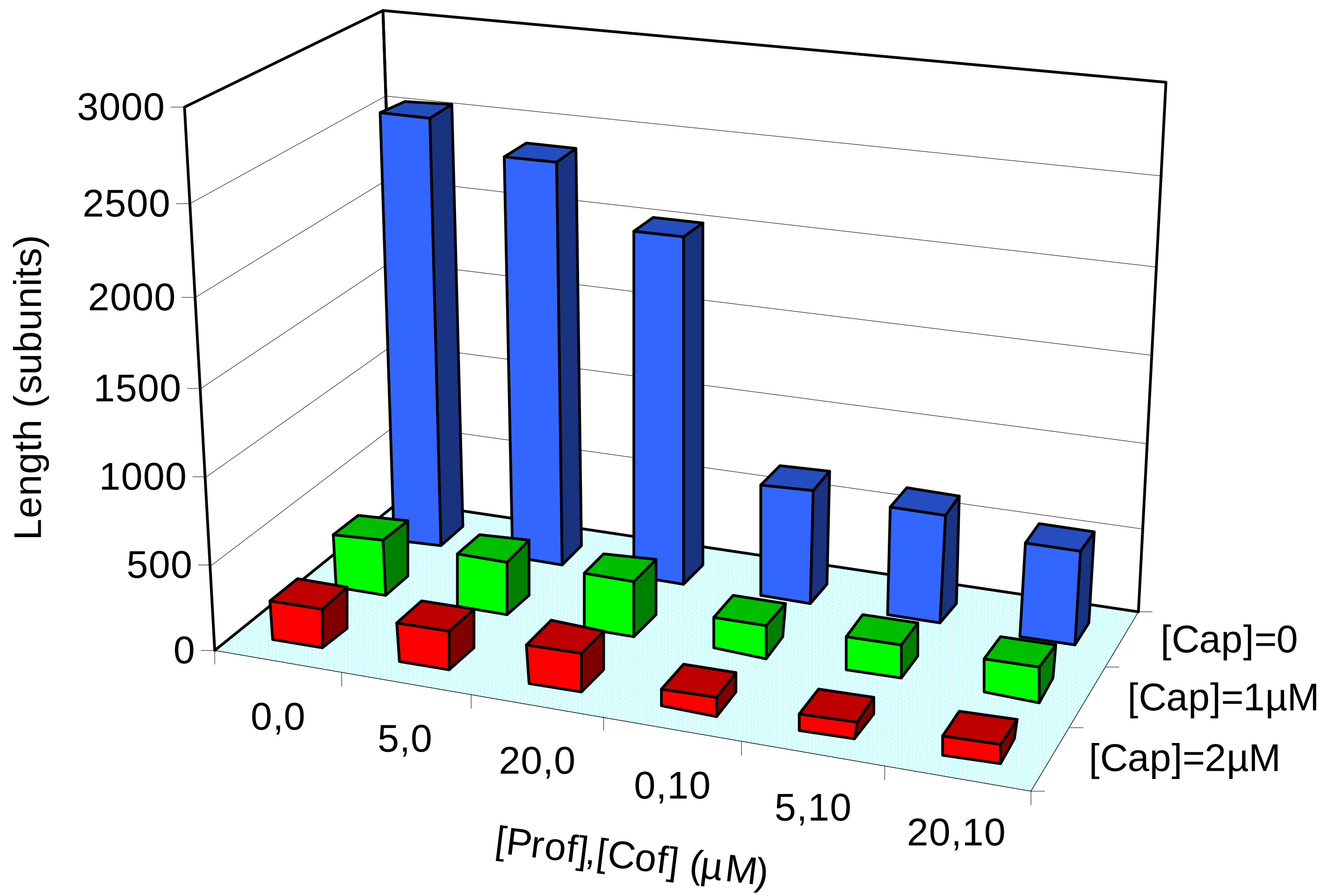
In the Cytosol




BarbedD
BarbedDPi
CBarbedT
Cap
CBarbedDPi
BarbedT
CBarbedD

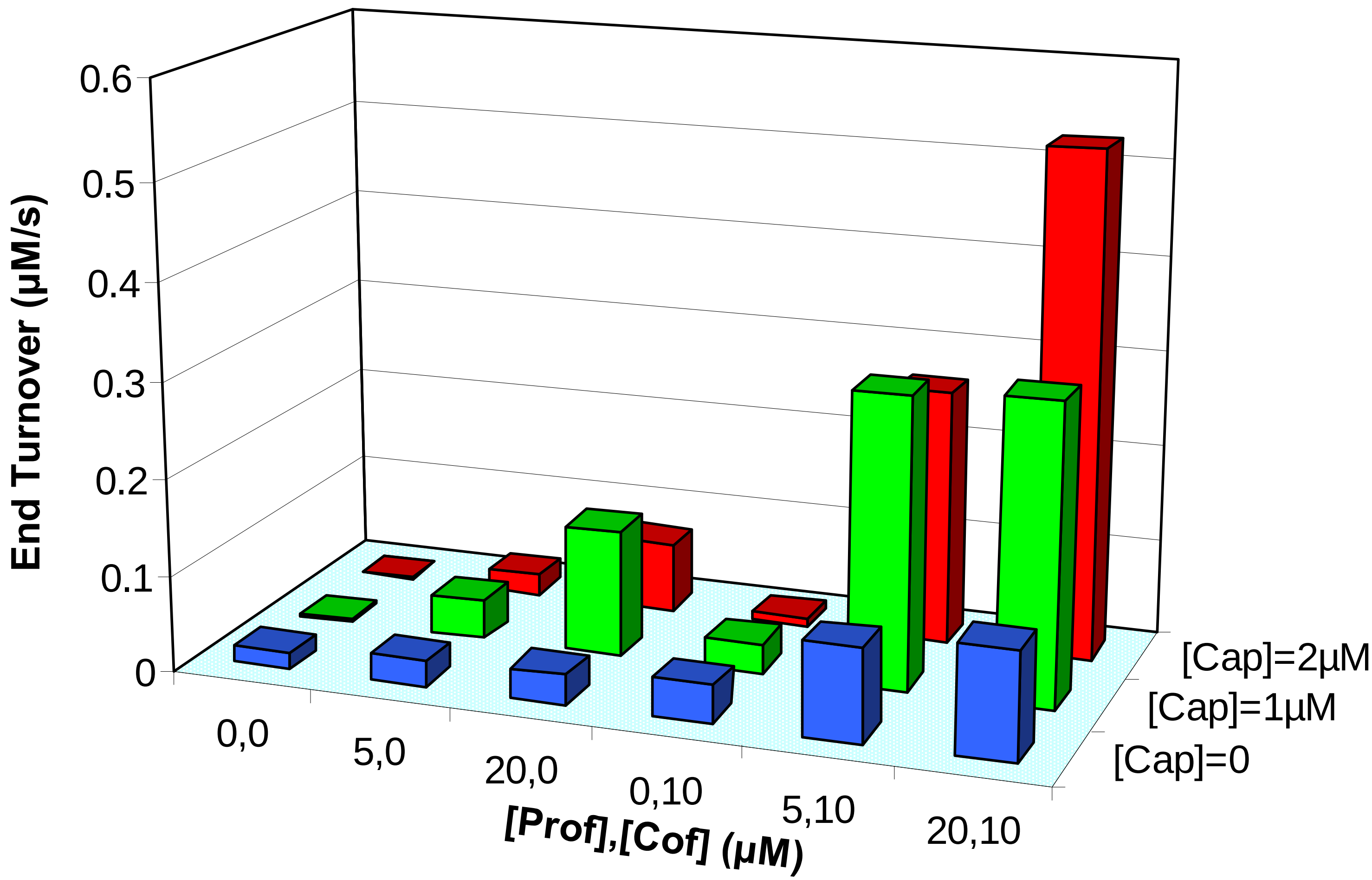
Steady State Lengths for 200µM Total Actin

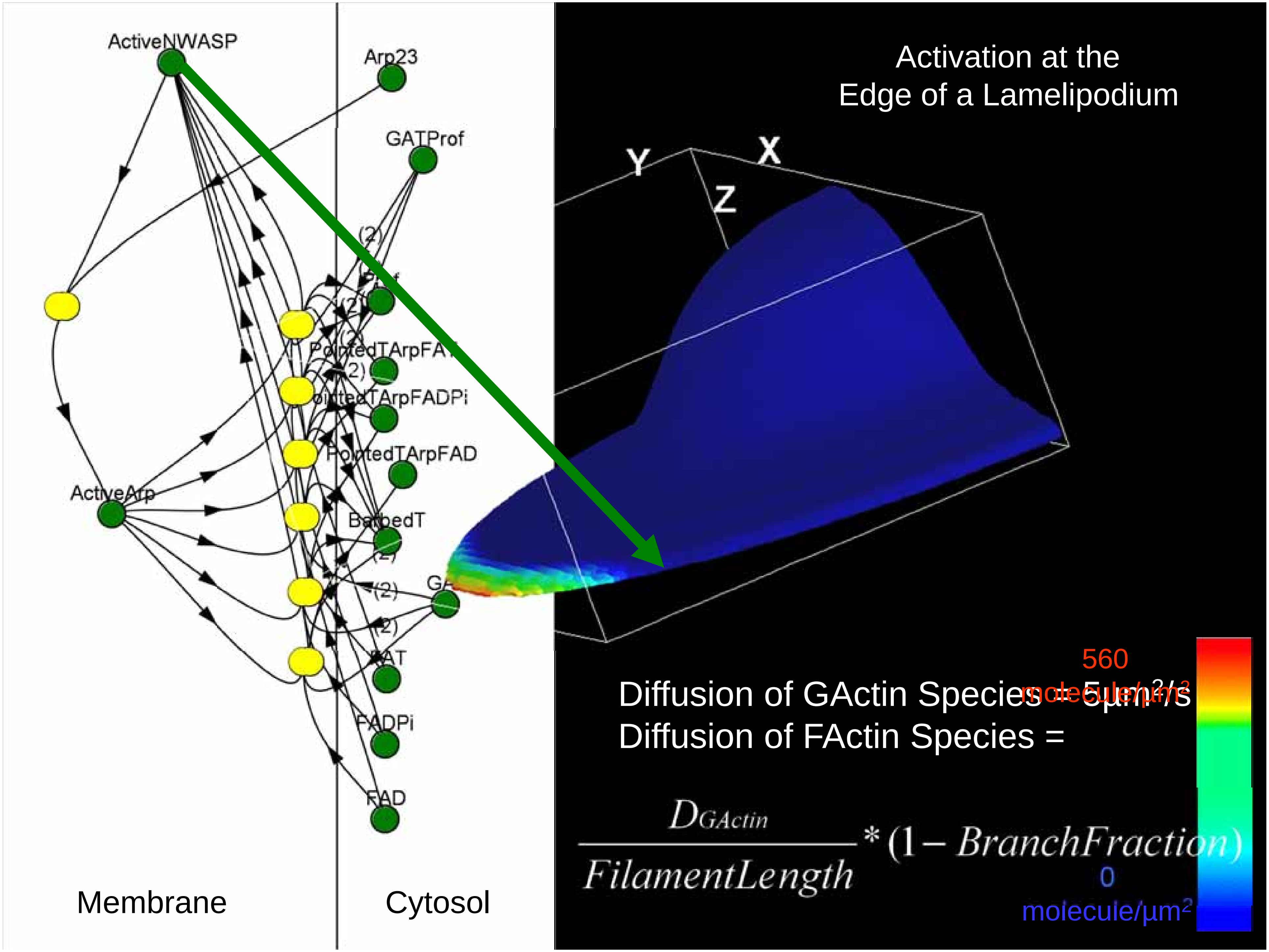
100 µM Thymosinß4, 0µM NWasp



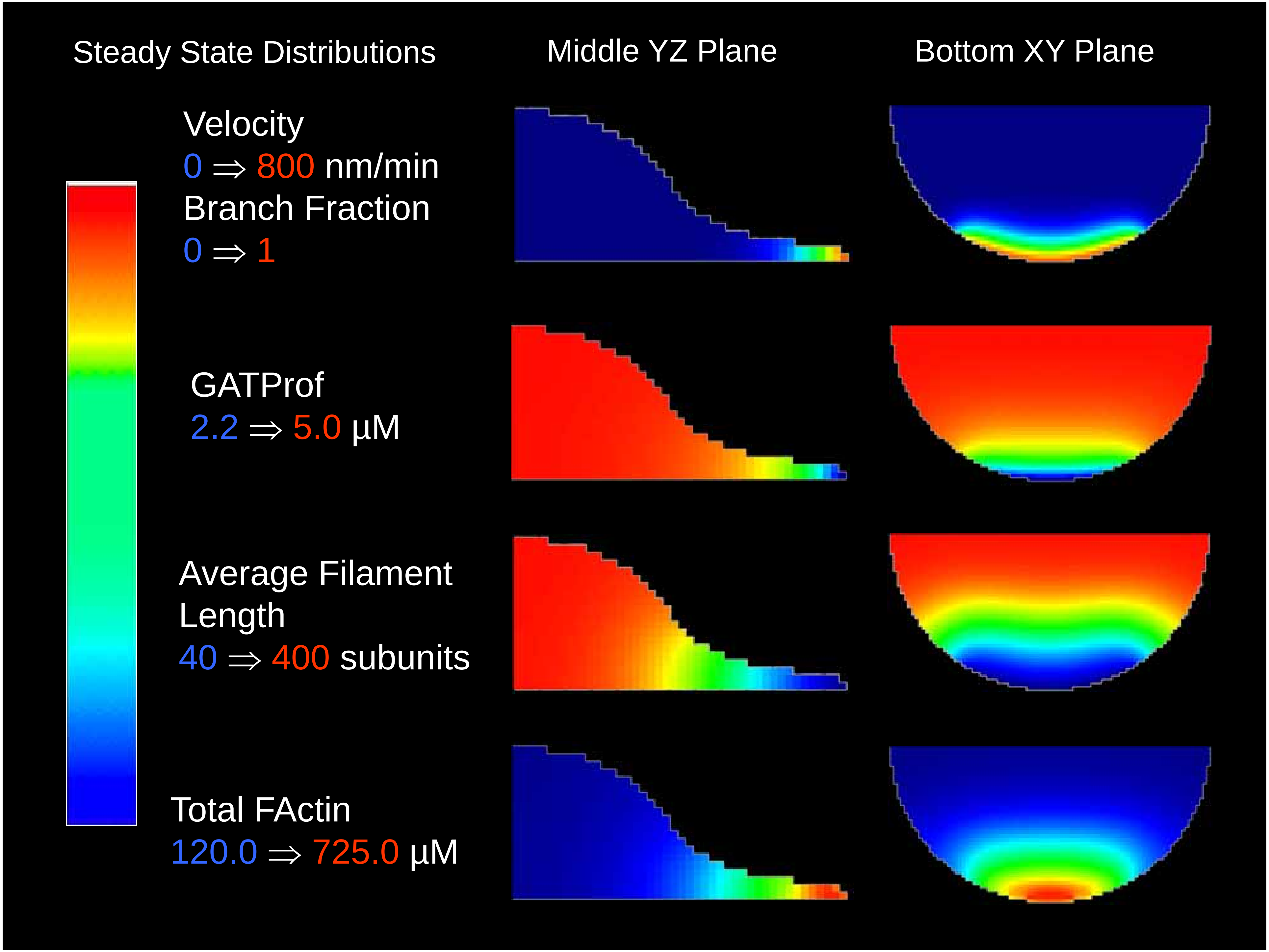
Filament Turnover for 200µM Total Actin

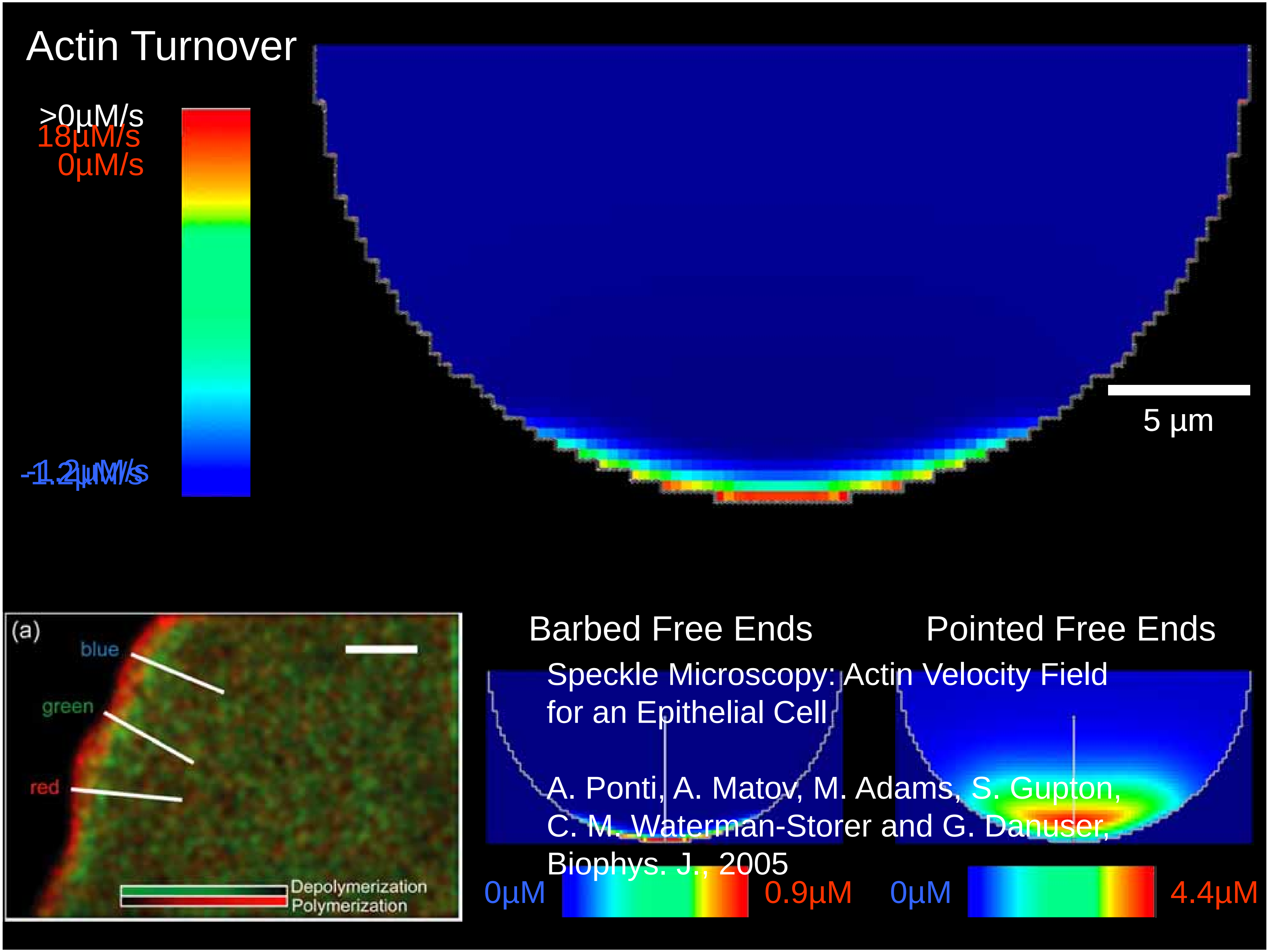
100 µM Thymosinß4, 0µM NWasp

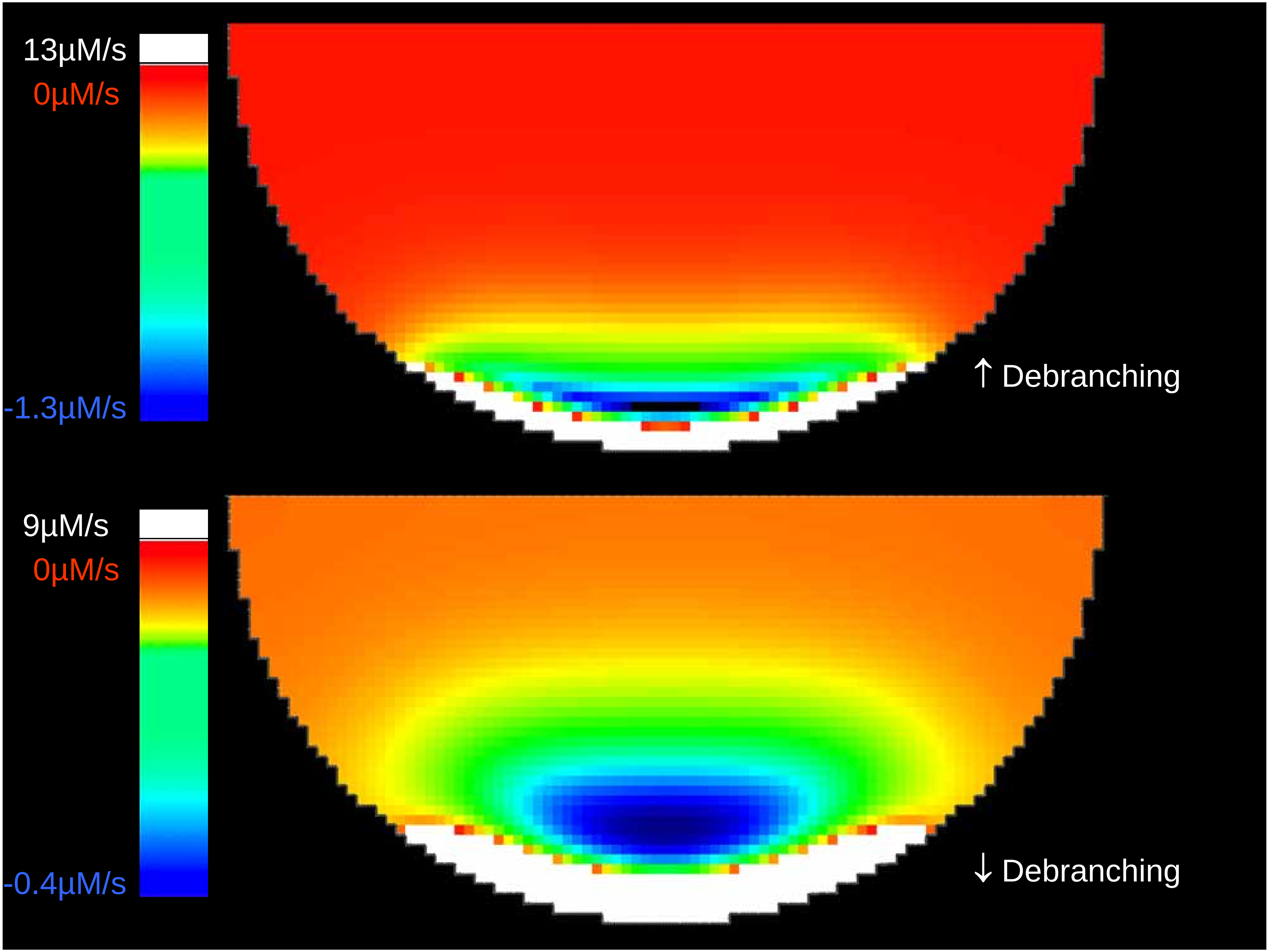




Activation at the
Edge of a Lamelipodium
ActiveNWASP
Arp23
GATProf
PointedTArpFAT
PointedTArpFADPi
PointedTArpFAD
ActiveArp
BarbedT
FAT
FADPi
FAD
(2)
Membrane
Cytosol
Y
X
Z
560
molecule/µm²
Diffusion of GActin Species = 5µm²/s
Diffusion of FActin Species =
$\frac{D_{GActin}}{FilamentLength} * (1 - BranchFraction)$
0
molecule/µm²

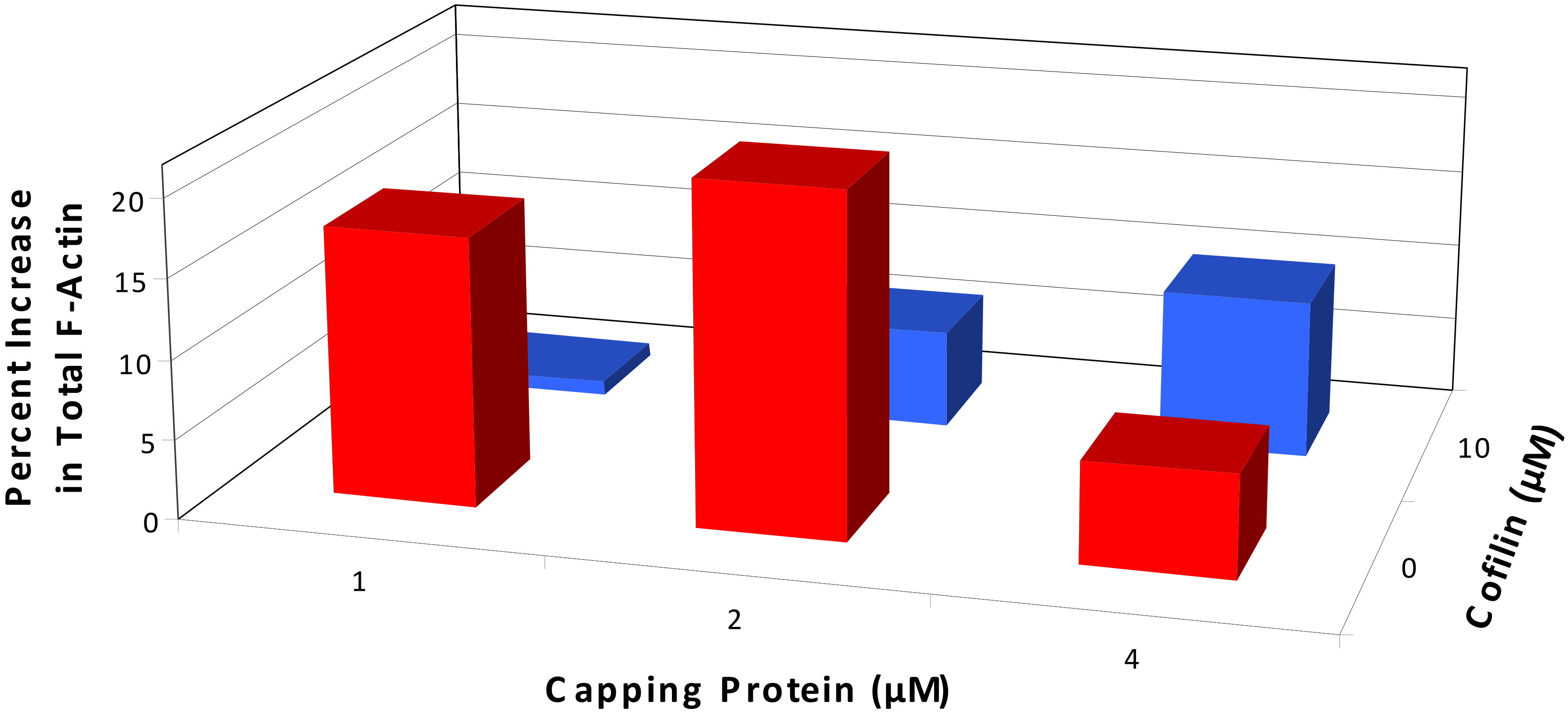

Steady State Distributions
Middle YZ Plane
Bottom XY Plane
Velocity
0 ⇒ 800 nm/min
Branch Fraction
0 ⇒ 1
GATProf
2.2 ⇒ 5.0 μM
Average Filament Length
40 ⇒ 400 subunits
Total FActin
120.0 ⇒ 725.0 μM


Actin Turnover
>0µM/s
18µM/s
0µM/s
-1.2µM/s
5 µm
(a)
blue
green
red
Depolymerization
Polymerization
Barbed Free Ends
Pointed Free Ends
Speckle Microscopy: Actin Velocity Field
for an Epithelial Cell
A. Ponti, A. Matov, M. Adams, S. Gupton,
C. M. Waterman-Storer and G. Danuser,
Biophys. J., 2005
0µM
0.9µM
0µM
4.4µM


13µM/s
0µM/s
-1.3µM/s
↑ Debranching
9µM/s
0µM/s
-0.4µM/s
↓ Debranching

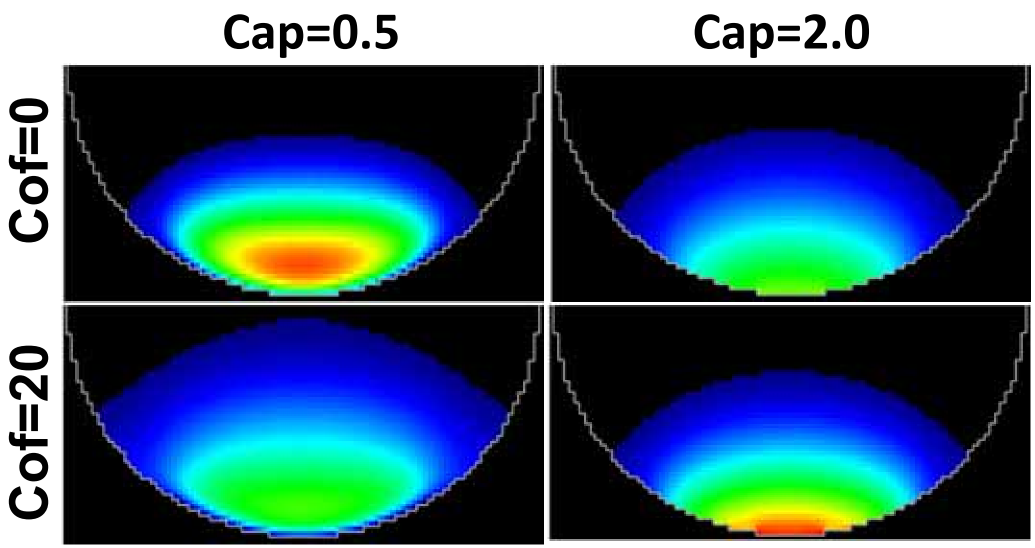
Does ADF/Cofilin Promote or Inhibit Actin Polymerization?

Sofya Borinskaya



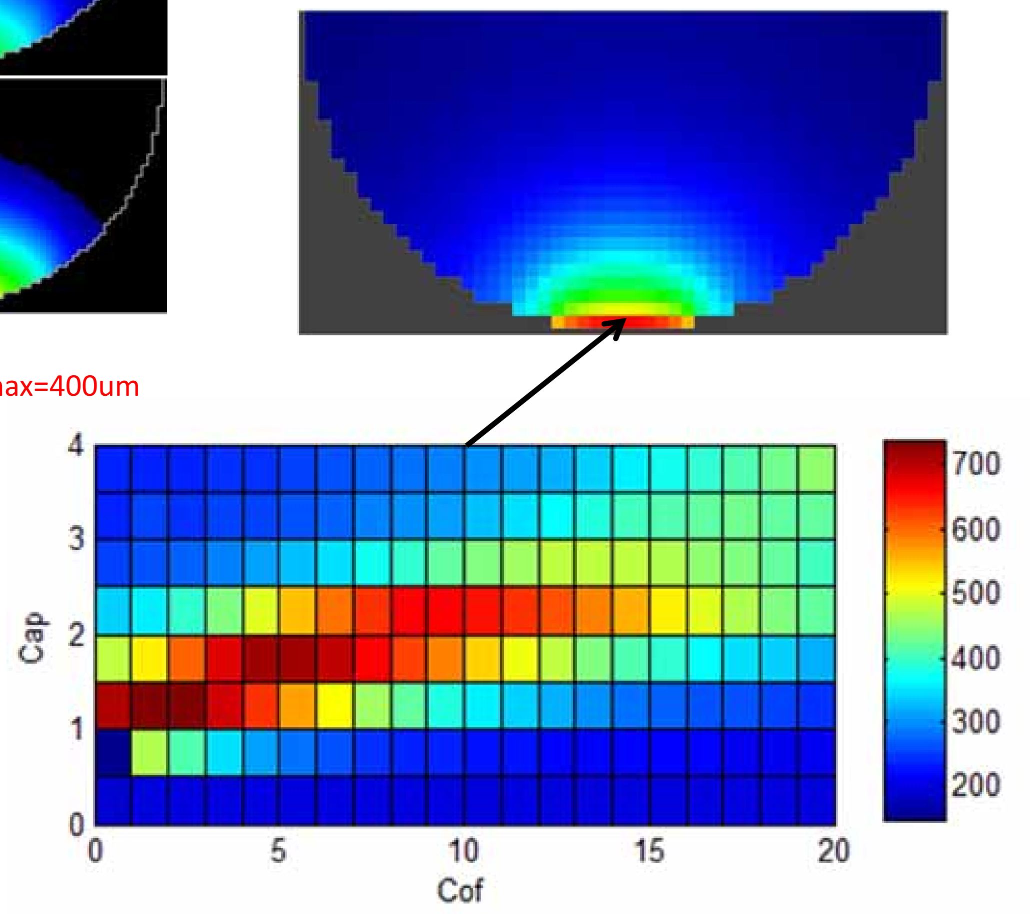
Interplay of cofilin and capping protein during localized activation of N-WASP

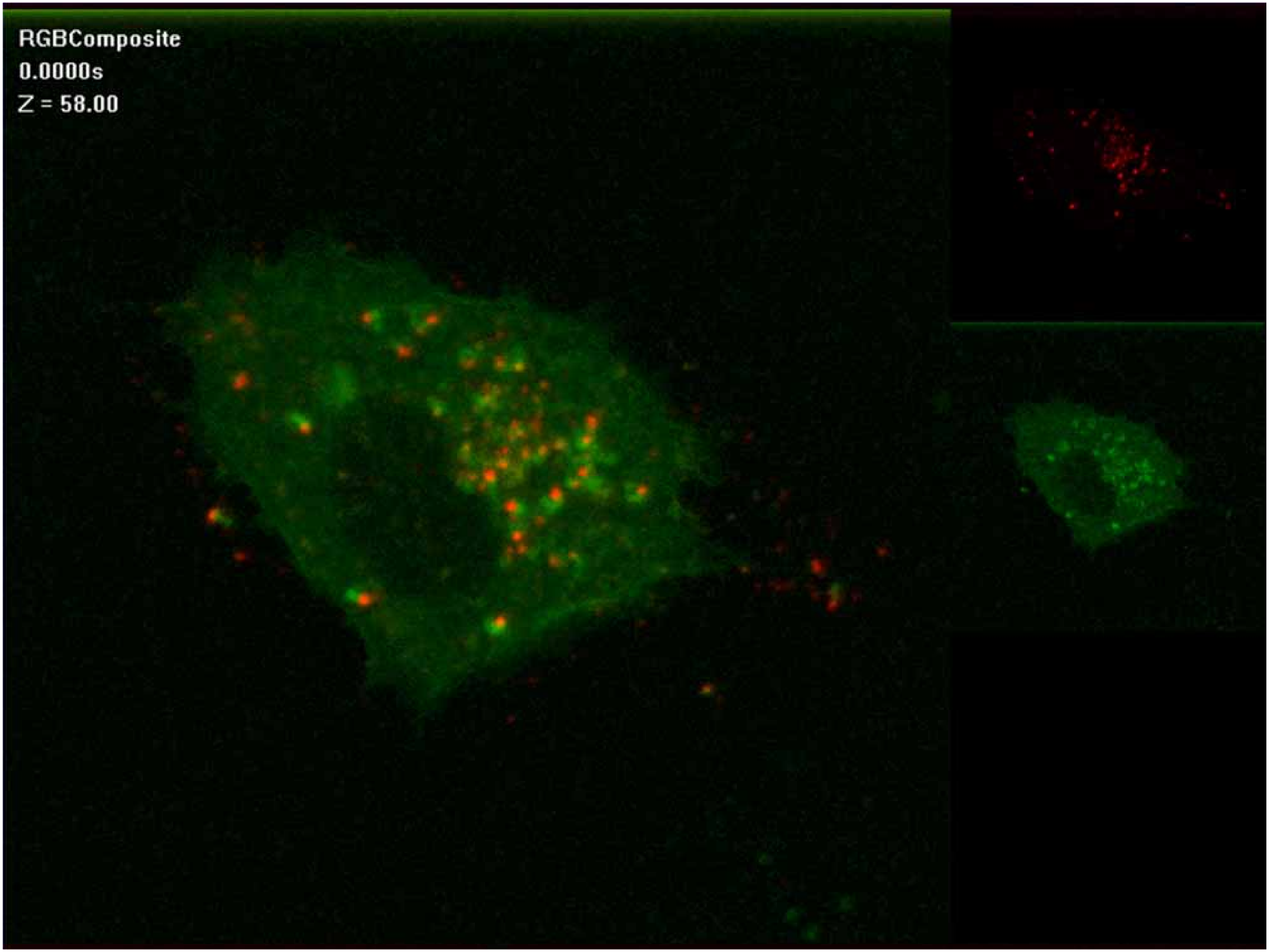
Sofya Borinskaya



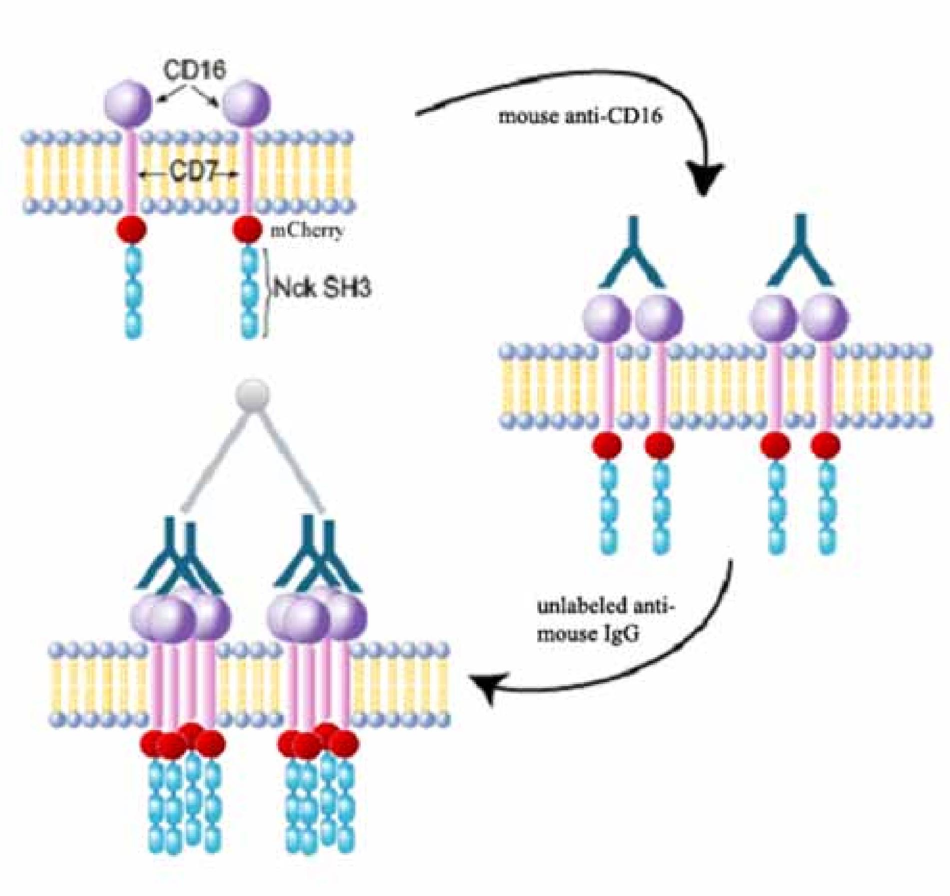
t=2000 s
F-actin min=180um max=300um

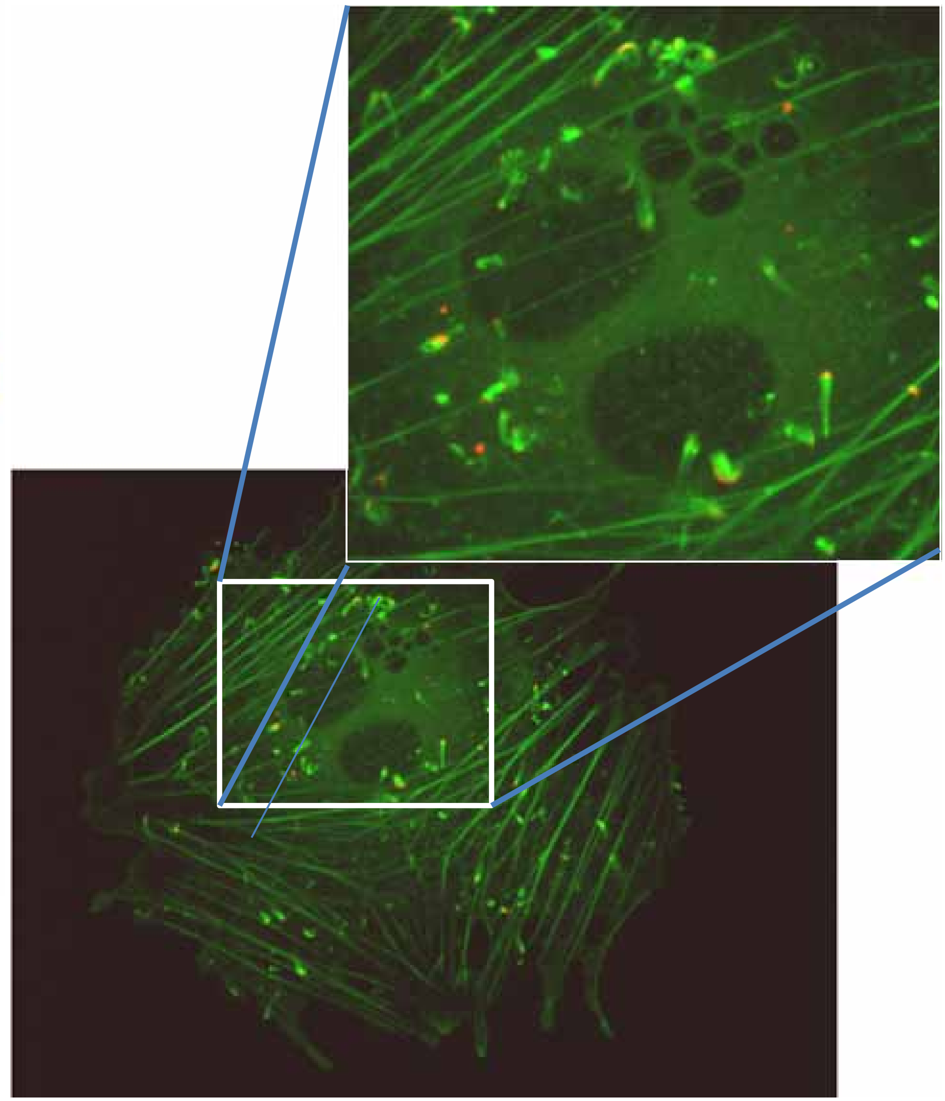
t=2000 s
F-actin min=150um max=400um

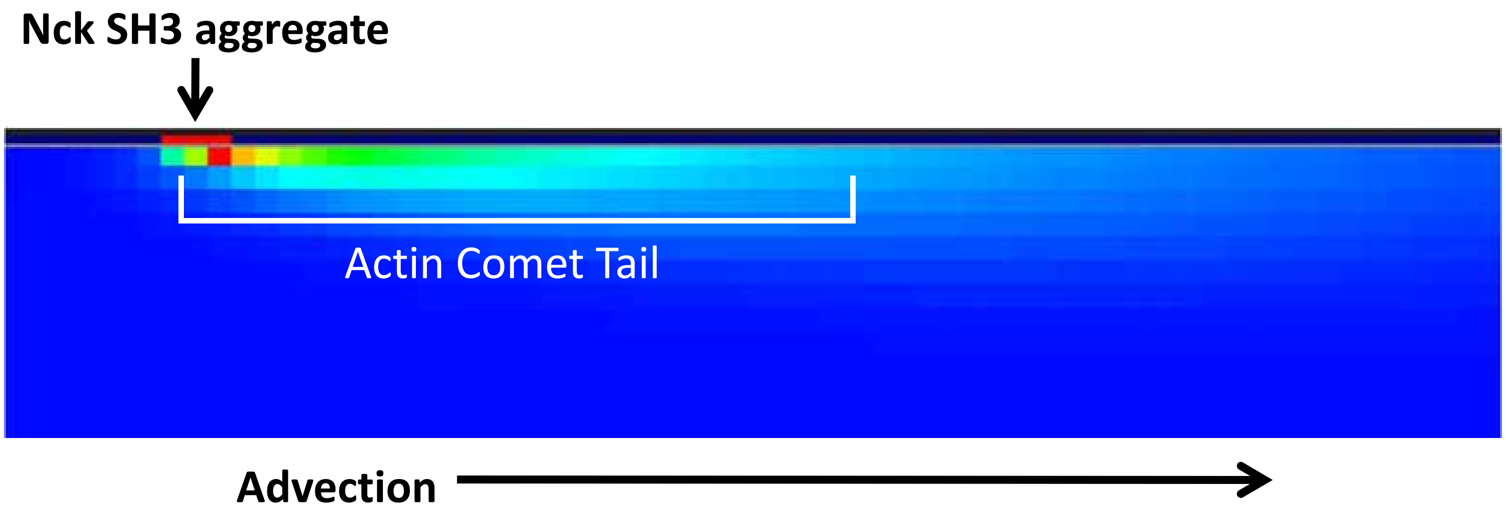



RGBComposite
0.0000s
Z = 58.00

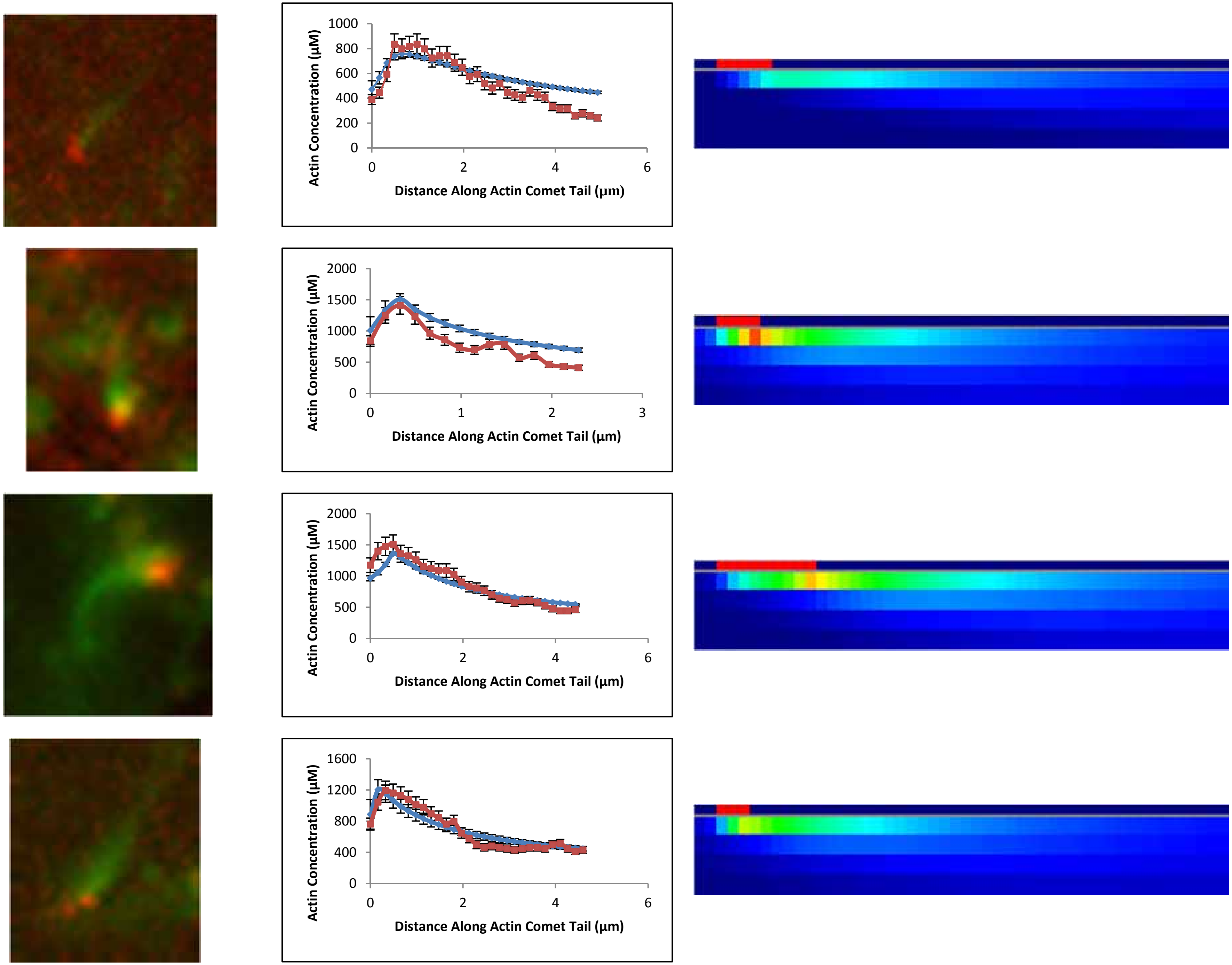
Pathway Manipulation



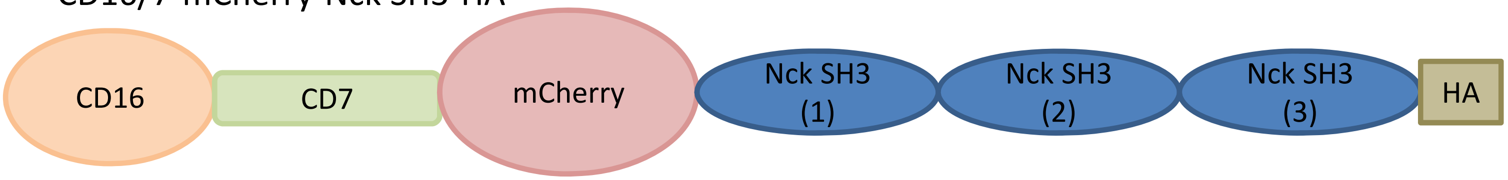



Nck SH3 aggregate
Actin Comet Tail
Advection

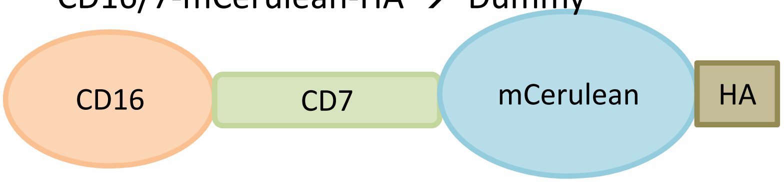
D

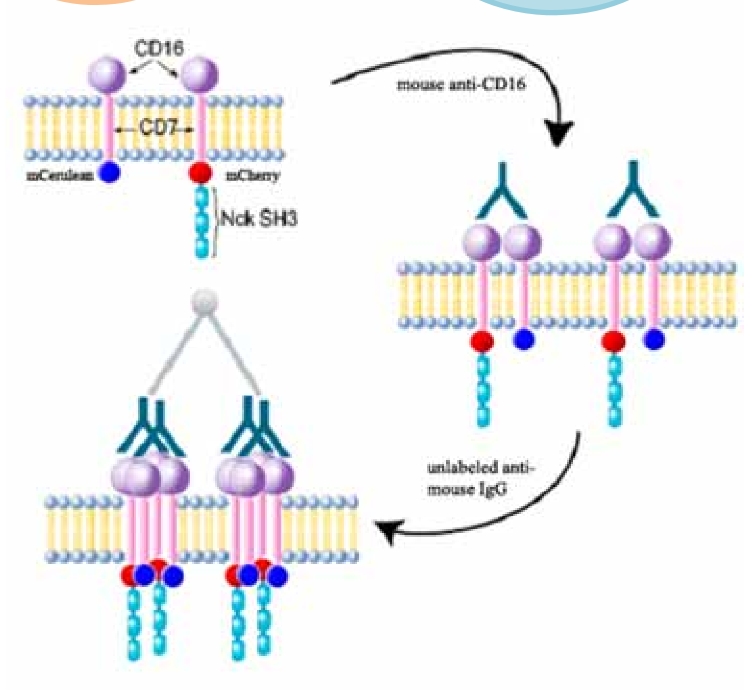

1000
800
600
400
200
0
0
2
4
6
Actin Concentration (µM)
Distance Along Actin Comet Tail (µm)
2000
1500
1000
500
0
0
1
2
3
Actin Concentration (µM)
Distance Along Actin Comet Tail (µm)
2000
1500
1000
500
0
0
2
4
6
Actin Concentration (µM)
Distance Along Actin Comet Tail (µm)
1600
1200
800
400
0
0
2
4
6
Actin Concentration (µM)
Distance Along Actin Comet Tail (µm)

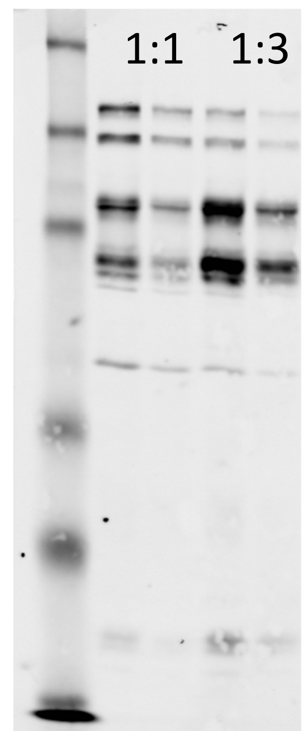
CD16/7-mCherry-Nck SH3-HA

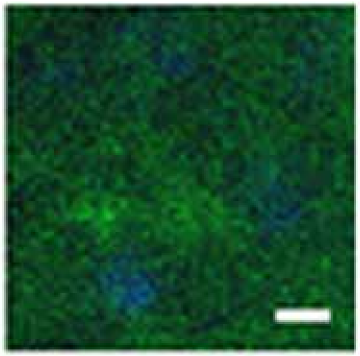


CD16/7-mCerulean-HA → 'Dummy'

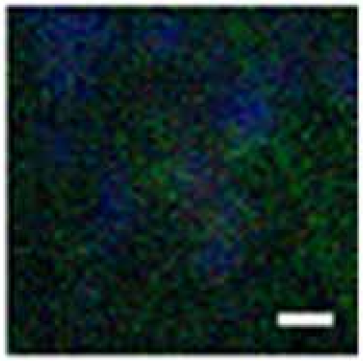




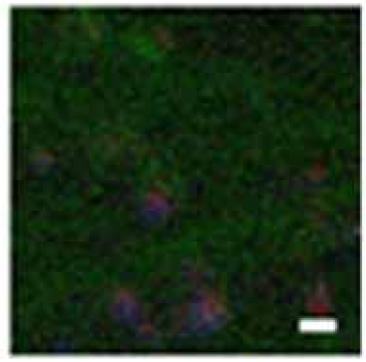




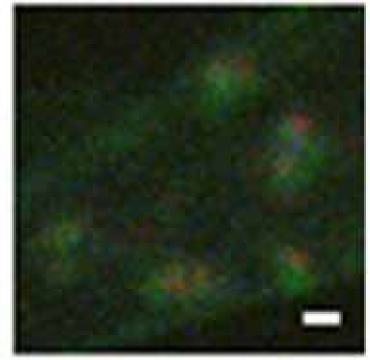
0% Nck SH3



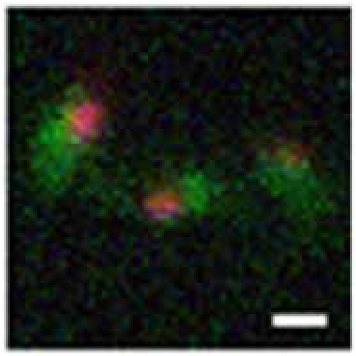
1-20% Nck SH3



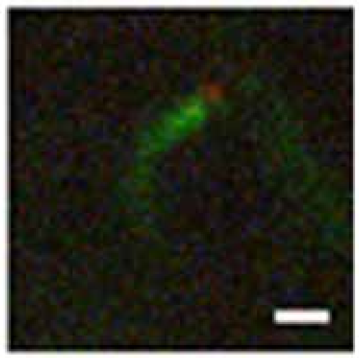
20-40% Nck SH3



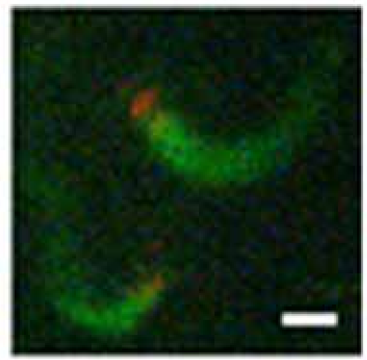
40-60% Nck SH3



60-80% Nck SH3



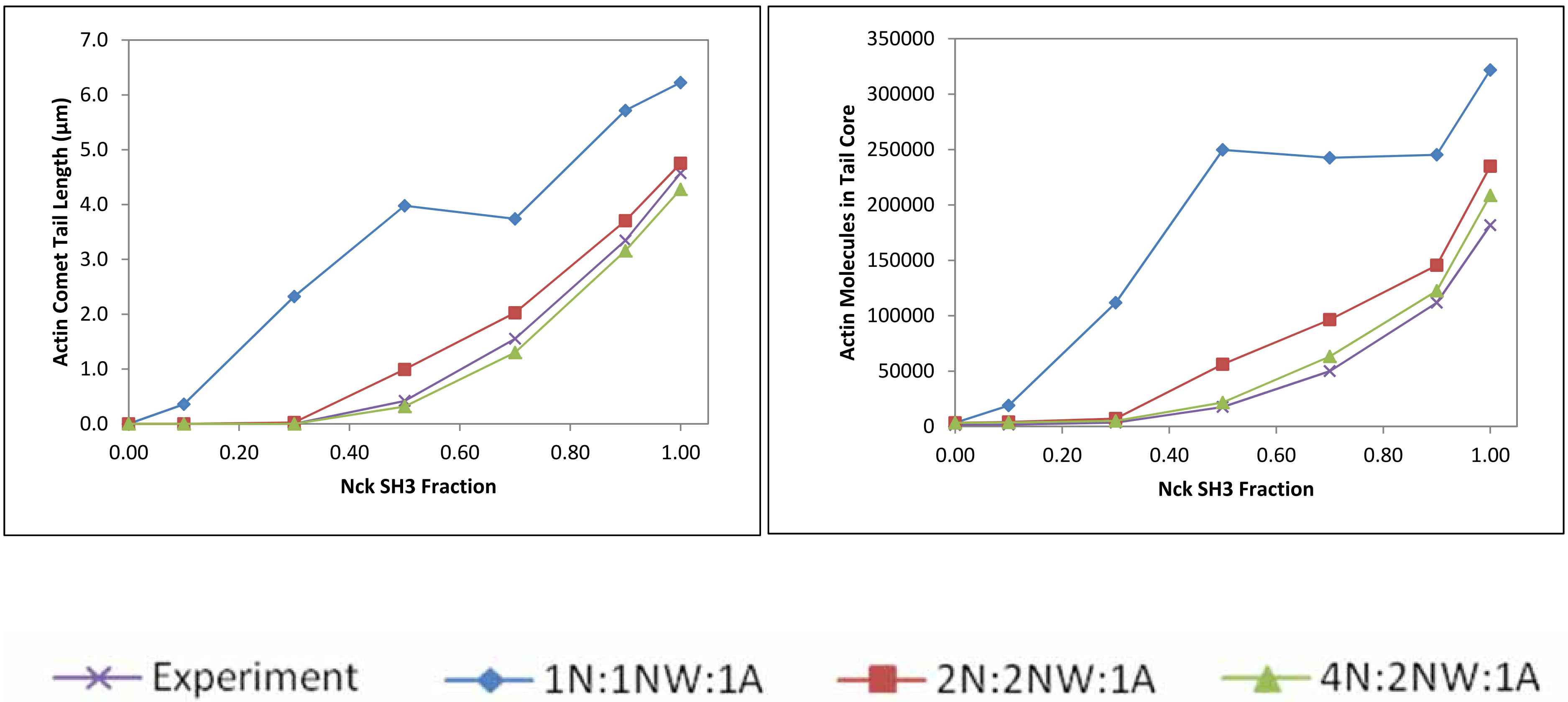
80-99% Nck SH3



100% Nck SH3

In all images: Green = Actin Blue = ‘dummy’ Red = Nck SH3

Titration of Nck SH3 density in aggregates reveals complex stoichiometry for Arp2/3 activation



Modeling of Chromophore Assisted Laser Inactivation of EGFP-Capping Protein

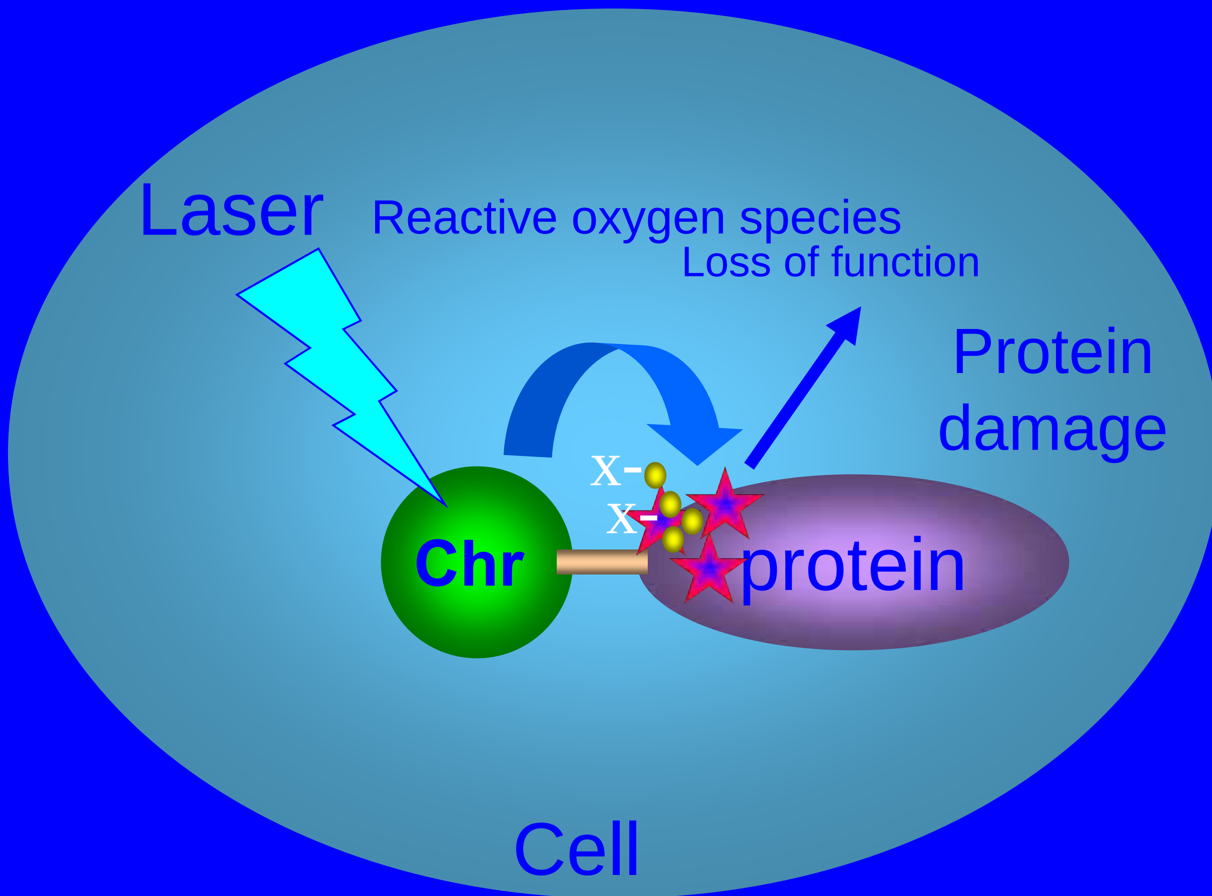
Maryna Kapustina

Eric Vitriol and Ken Jacobson

Cell and Developmental Biology, UNC Chapel Hill

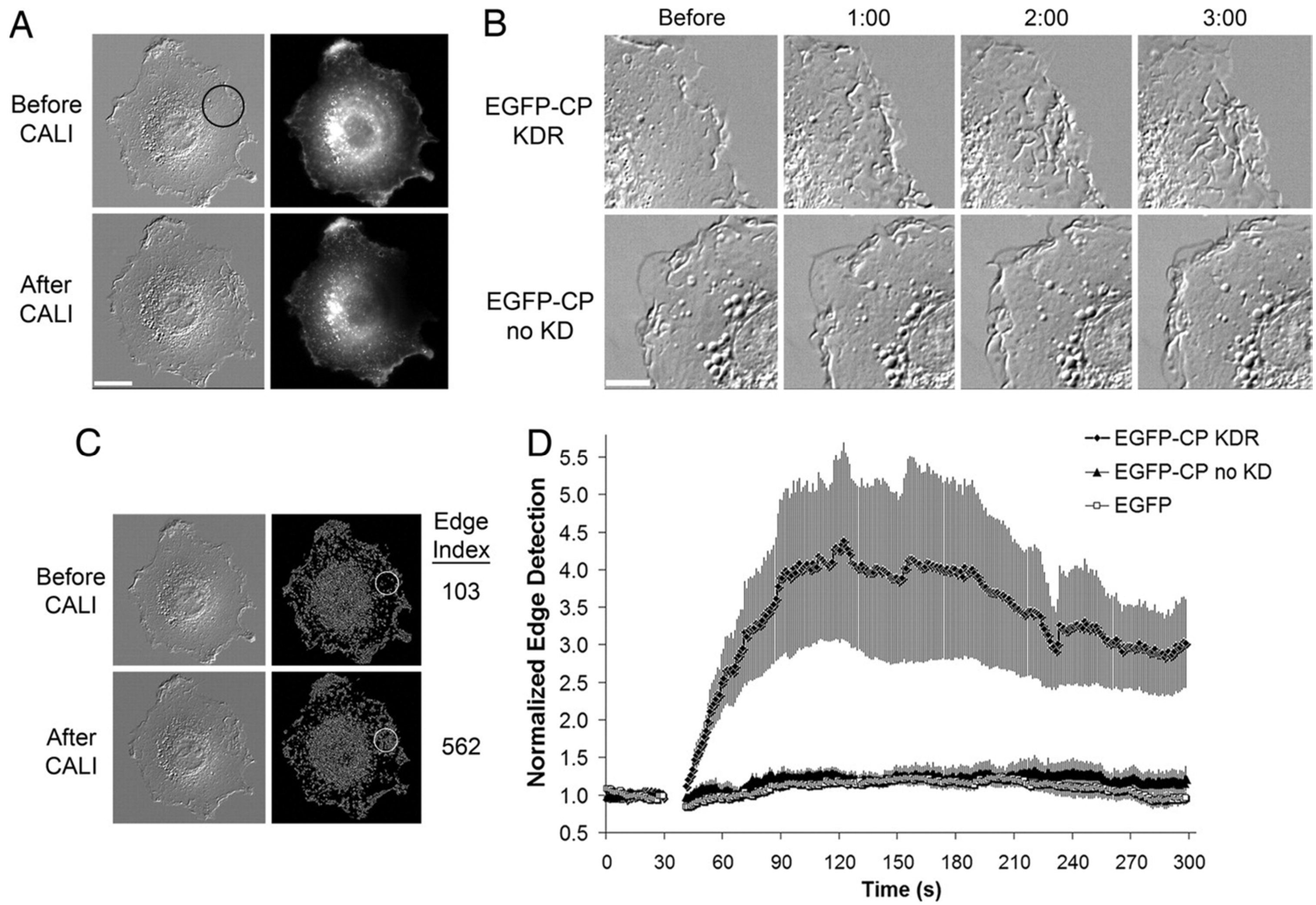
Kapustina, M., E. Vitriol, T.C. Elston, L.M. Loew, and K. Jacobson. 2010. Modeling capping protein FRAP and CALI experiments reveals in vivo regulation of actin dynamics. Cytoskeleton. 67:519-534.

CALI Mechanism

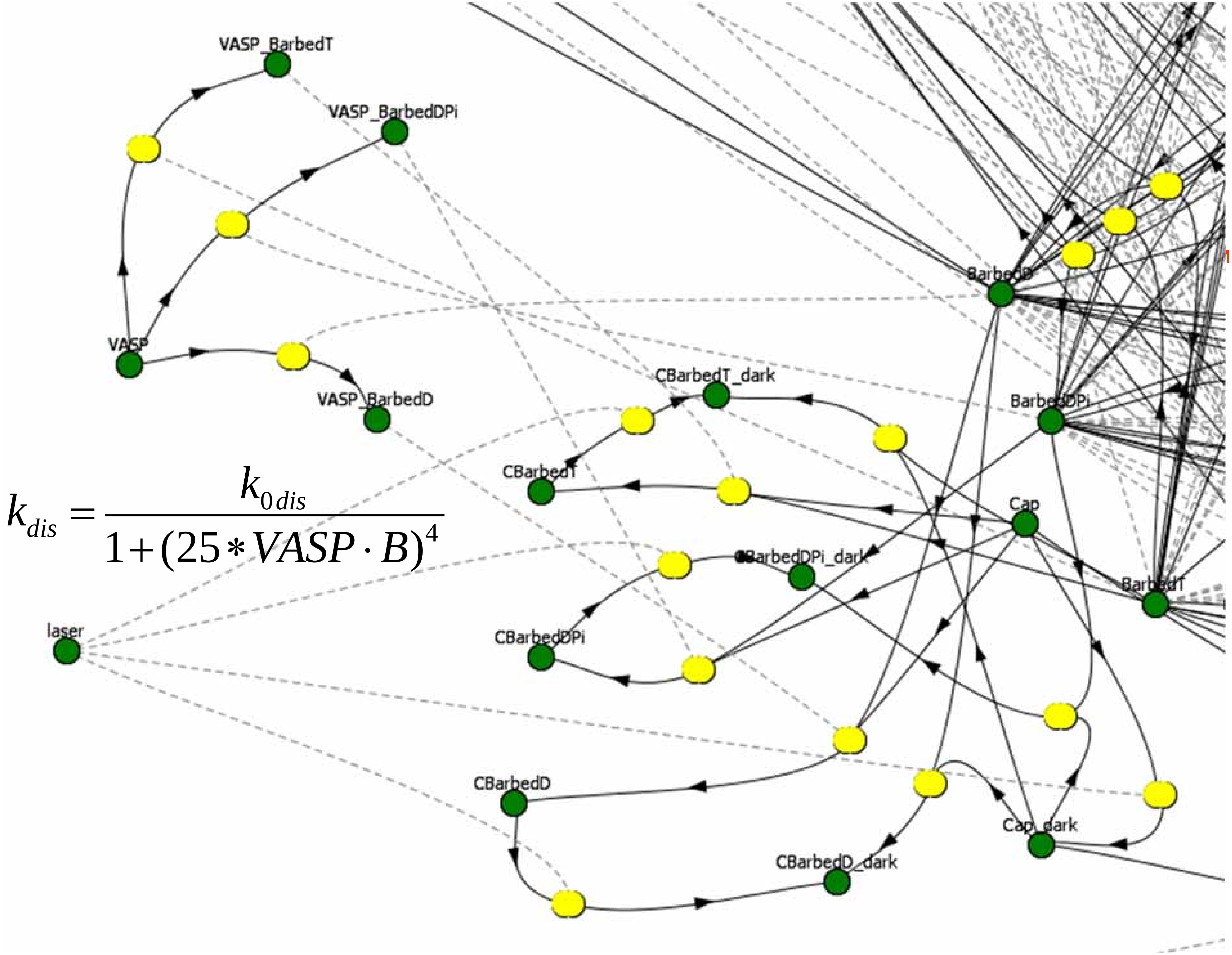


• Chromophore excitation leads to production of free radicals
• Free radicals are highly destructive, causing protein damage
- short half-life (nm destruction radius)
• Potential for local, instantaneous inactivation of adjacent protein

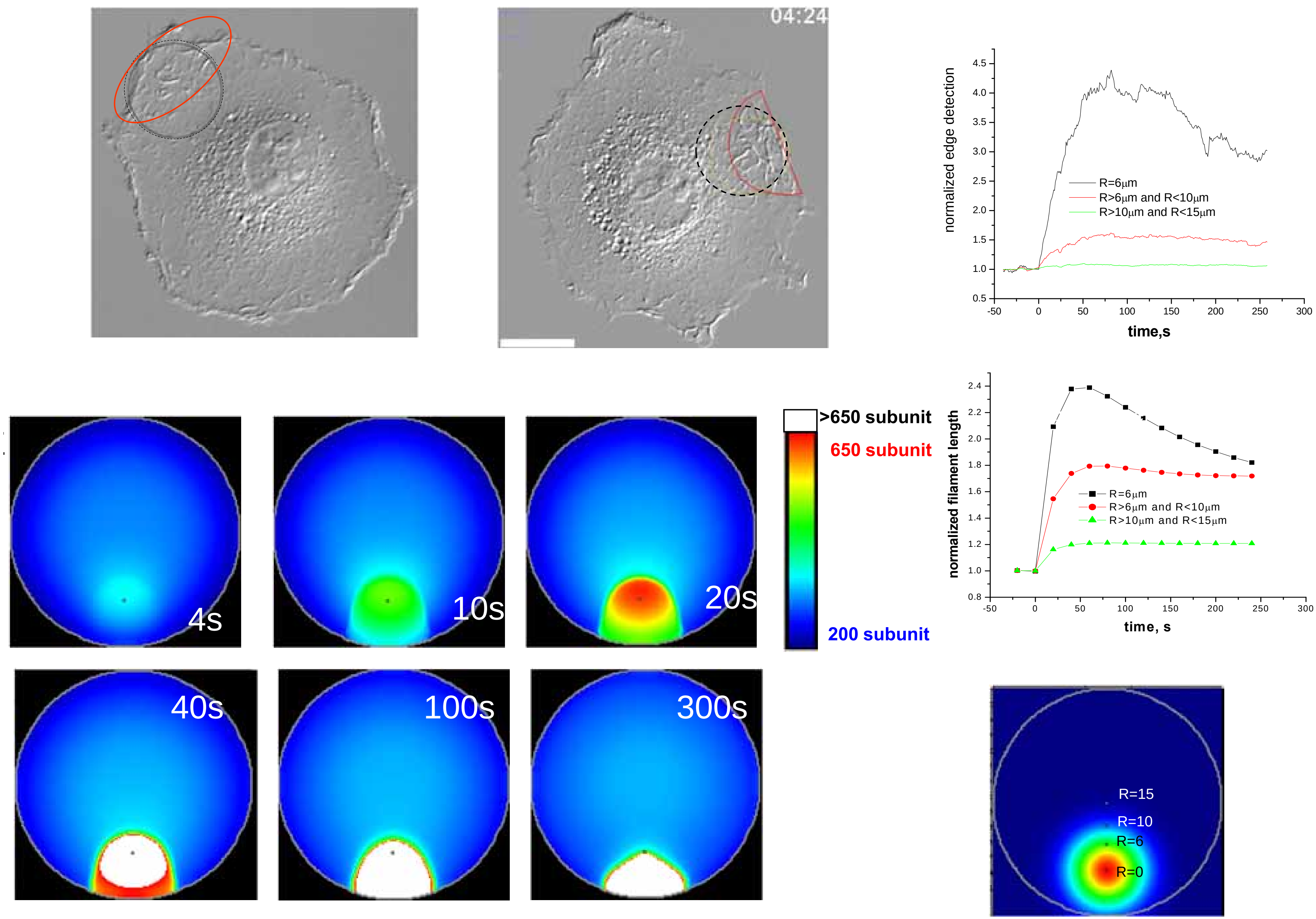
CALI of EGFP-CP induces the formation of dorsal ruffles and filopodia only in the knockdown/rescue background



Vitriol E. A. et.al. PNAS 2007;104:6702-6707



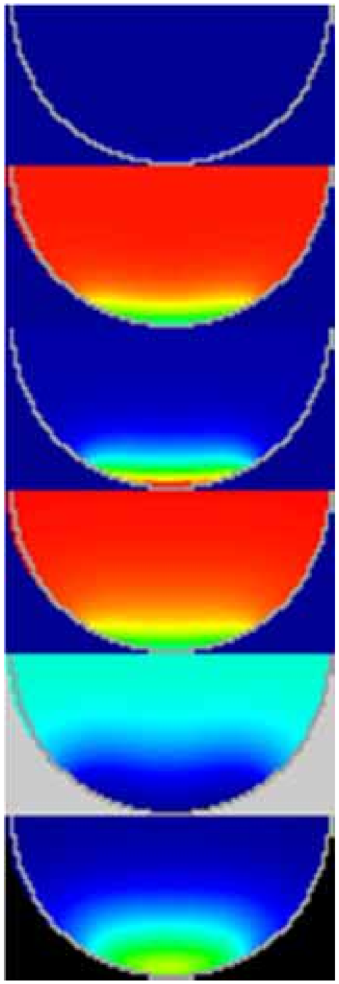
$$k_{dis} = \frac{k_{0\,dis}}{1 + (25 * VASP \cdot B)^4}$$



04:24
normalized edge detection
time,s
R=6μm
R>6μm and R<10μm
R>10μm and R<15μm
4s
10s
20s
40s
100s
300s
>650 subunit
650 subunit
200 subunit
normalized filament length
time, s
R=6μm
R>6μm and R<10μm
R>10μm and R<15μm
R=15
R=10
R=6
R=0

Virtual CALI on Lamelipodium

- BarbedEnds 0 11 µM
- Free Cap 0 0.21 µM
- CappedBarbedEnds 0 15.3 µM
- G-Actin-ATP-Prof 0 5.0 µM
- FilamentLength 42 610 subunits
- Total F-Actin 115 990 µM



100s; total actin, 200µM; thymosin-B4, 100µM; profilin, 10µM; capping protein, 1µM; Arp2/3, 1µM

Conclusions

- The sharp transition between actin assembly and disassembly in the lamelipdoium emerges from the backflow and dissociation of Ar2/3 branches
- The model reveals the interplay of ADF/cofilin and capping protein in Arp2/3-dependent actin polymerization
- Analysis of Virtual CALI reveals a cooperative VASP activity for the assembly of filament bundles
- Actin distributions in Nck SH3 domain comet tails are determined by both cluster size and comet velocity
- The nonlinear SH3 domain density-dependence of actin comets can be reconciled by the stoichiometry of the signaling molecules recruited by the Nck adaptor
- This “open” VCell model can serve as a basis for hypothesis generation and as a module for the response of actin to cell signaling