Mesoscopic Physics of Motile Protrusions in Eukaryotic Cells

Garegin Papoian
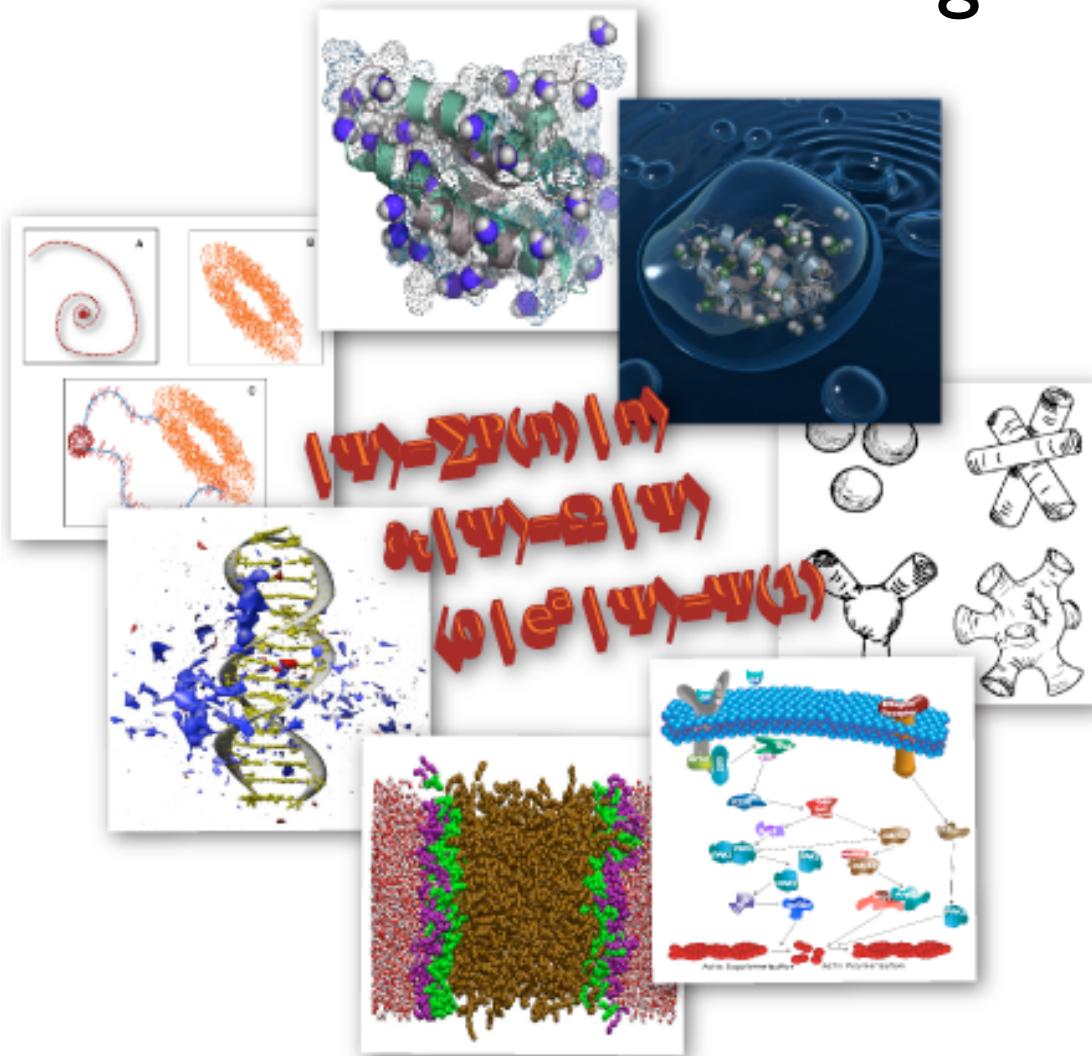
Department of Chemistry and Biochemistry

&

Institute for Physical Science and Technology

University of Maryland

# Cell's Cytoskeleton at the Leading Edge

**Wadsworth lab at the University of Massachusetts**
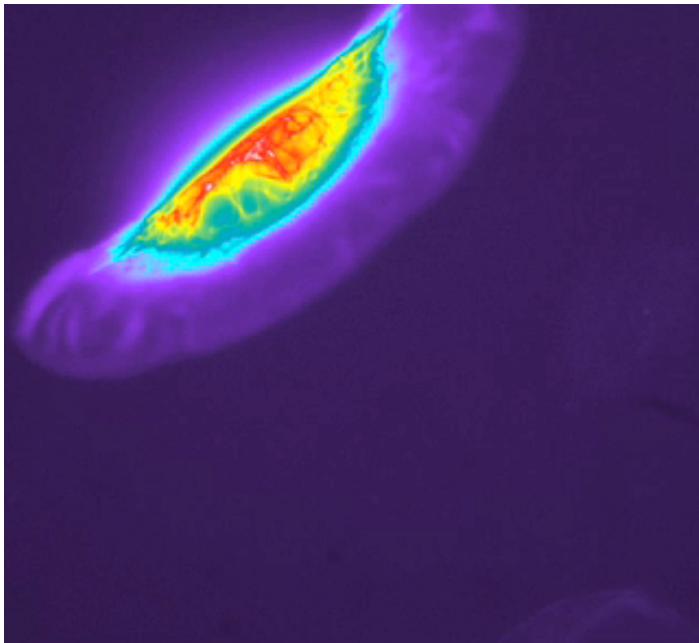
Motile Cell
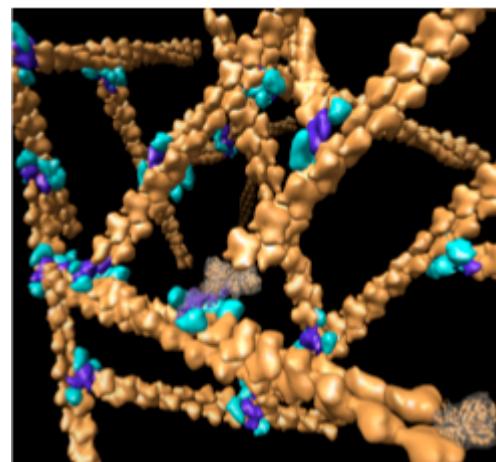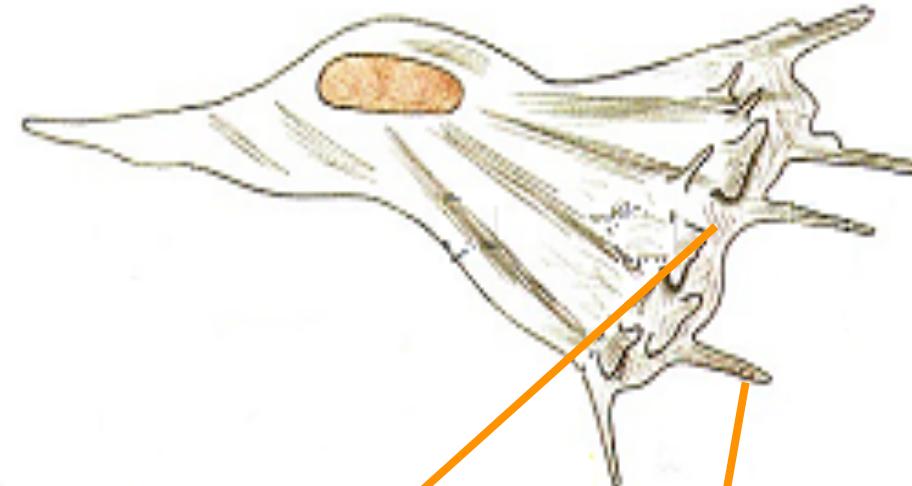
Lamellipodial Actin (3D)
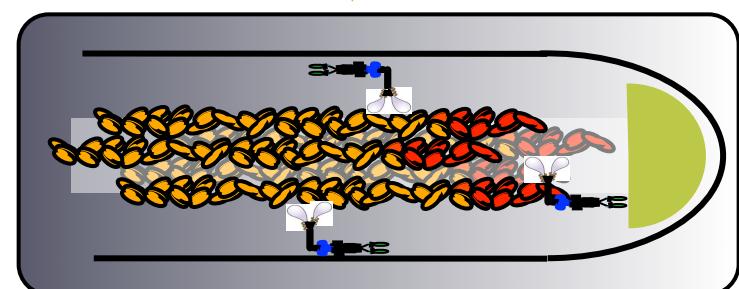
Filopodial Actin (1D)

Dendritic nucleation/Array treadmilling model

1. Extracellular stimuli

2. Produce active GTPases & PIP2

3. Activate WASp/Scar

4. Activate Arp2/3 complex to initiate new filaments

Activated GTPases & PIP2

WASp/Scar

Arp2/3 complex

6. Growing filaments push membrane forward

70°

5. Barbed ends elongate

7. Capping protein terminates elongation

PAK

12. LIM-kinase inhibits ADF/cofilin

8. Aging

9. ADF/cofilin severs & depolymerizes ADP- filaments

11. Pool of ATP-actin bound to profilin

ADP-actin

10. Profilin catalyzes exchange of ADP for ATP

Pollard and Borisy, Cell 112, 453 (2003).

Regulation of force generation is complicated!

Actin filaments and monomer diffusion

G-actin
45kDa

ATP

"pointed end"

"barbed end"

-

+

F-actin

$2\delta =$ 5.4nm   37nm
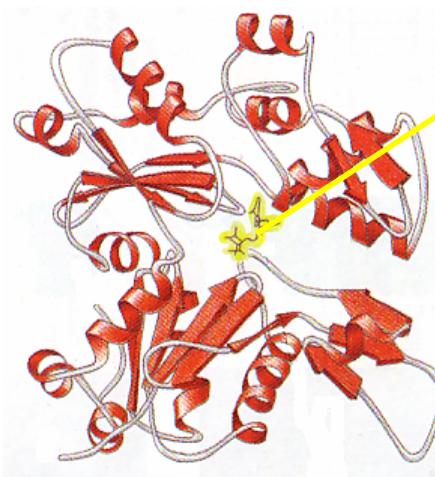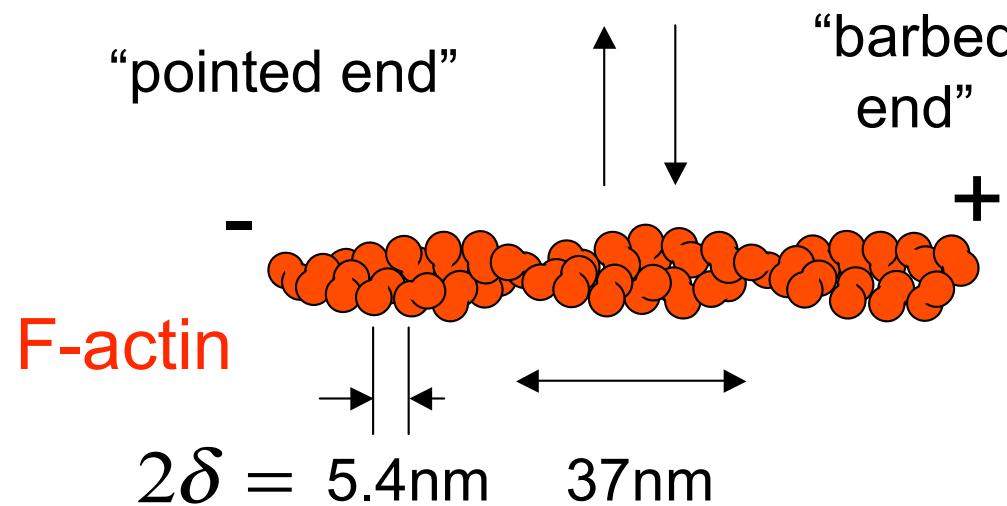
Some parameter values

|	
Persistence length	$15\mu m$
Buckling length	$120nm\,(10pN)$
Diffusion rate	$5\mu m^2 / s$
Bulk concentration	$10\mu M$
Polymerization rate	$10\mu M^{-1}s^{-1}$

Mogilner and B. Rubinstein, *Biophys. J.* 89, 782

3D Active Mesh within Lamellipodia
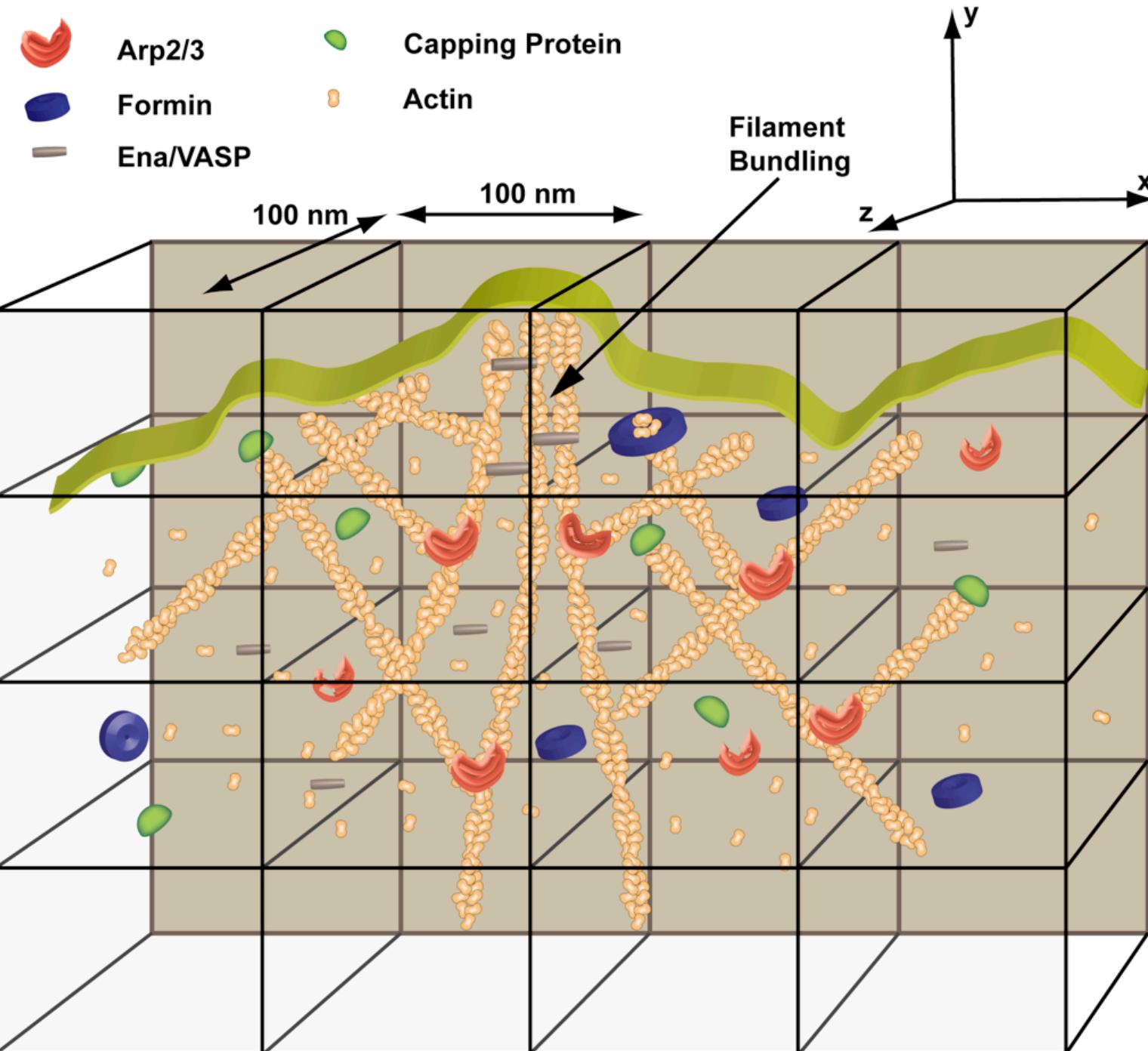
Stochastic simulations of lamellipodia protrusion

- Simulation region is divided into compartments.

- Diffusion (Actin, Capping protein, Arp2/3) between compartments.

- Chemical reactions in compartments:

  - Polymerization, Depolymerization, Capping, Branching...

- Monte Carlo algorithm to generate stochastic trajectories
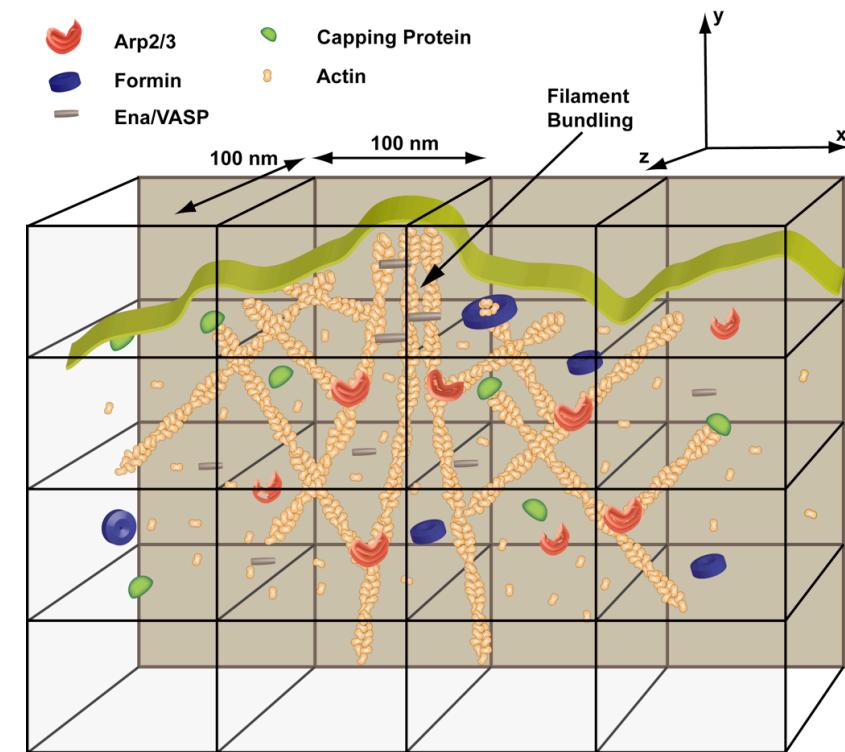
✏ L. Hu and G. A. Papoian, **Biophys. J.**; *2010, 98,1375*

Filaments and membrane

- Filaments are assumed to be straight and rigid.
- Leading edge membrane is modeled as a 1-D curve $x=x(y,z)=h(y)$ due to the flatness of the lamellipodia: ~200nm (z) compared to micron size of the other direction (y).

The membrane is modeled as an elastic sheet under tension which also resists bending

Steric repulsion between the filament tips and the membrane
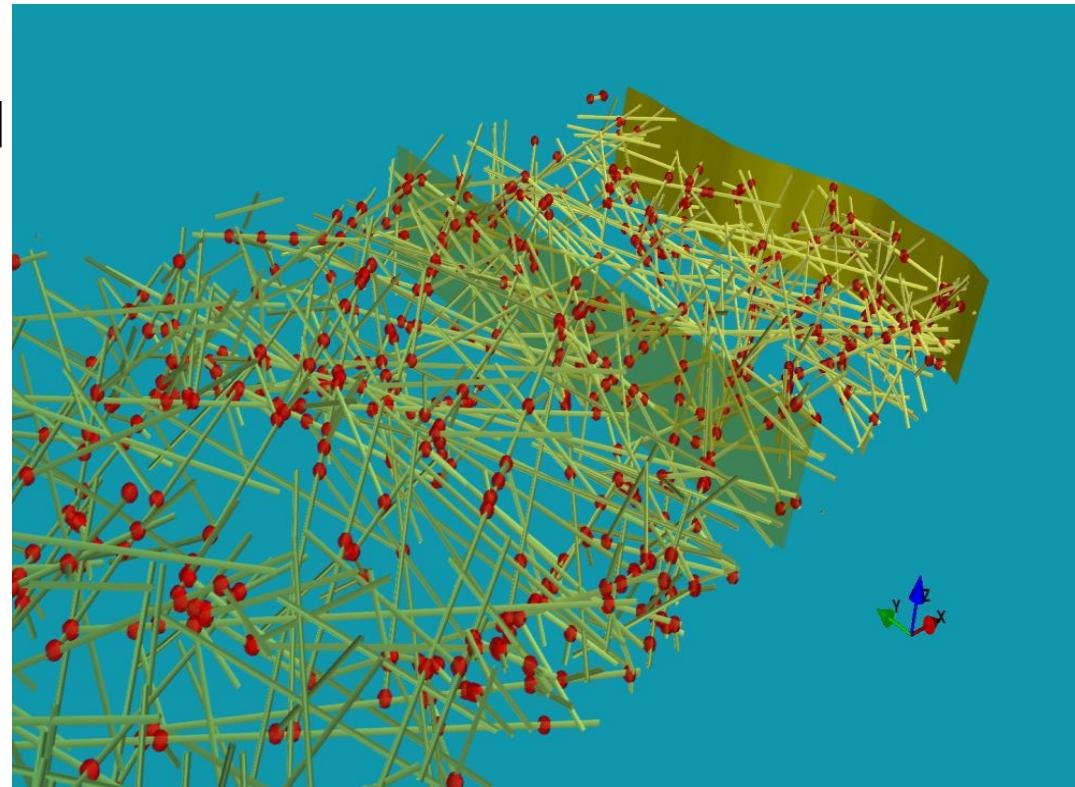
Stochastic Growth of a Lamellipodium

- **Tubes** indicate growing actin filaments

- **Red** spheres indicate Arp2/3 nucleation points

- Diffusing monomeric species are not shown (actin, Arp2/3, and capping proteins)

- Mechano-chemical couplings between the membrane and the filament growth

Capping protein enhances motility

- Capping proteins block the polymerization of actin filaments.
- However, capping protein can enhance motility. The mechanism?
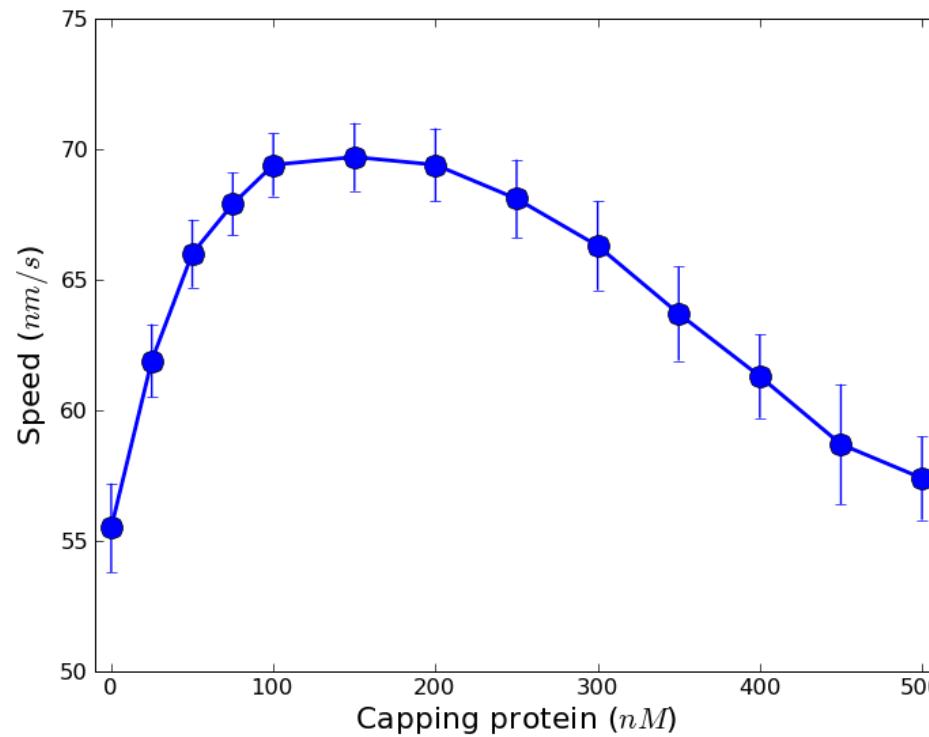
Experimental studies on capping protein promotes motility:
Carlier and Pantaloni, JMB 269, 459 (1997).
Loisel et al., Nature 401, 613 (1999).
Akin and Mullins, Cell 133, 841 (2008).

Numerous Long Filopodia Grow in *HeLa* Cells

00:00

- Biological role of filopodia
  - Guiding cell movement
  - Embryonic development, wound healing, cancer spreading
- Filopodial structure
  - Parallel actin filaments, enveloped by a membrane
  - Cross-linking proteins
  - Tip protein complex

Our Computational Model for Filopodia
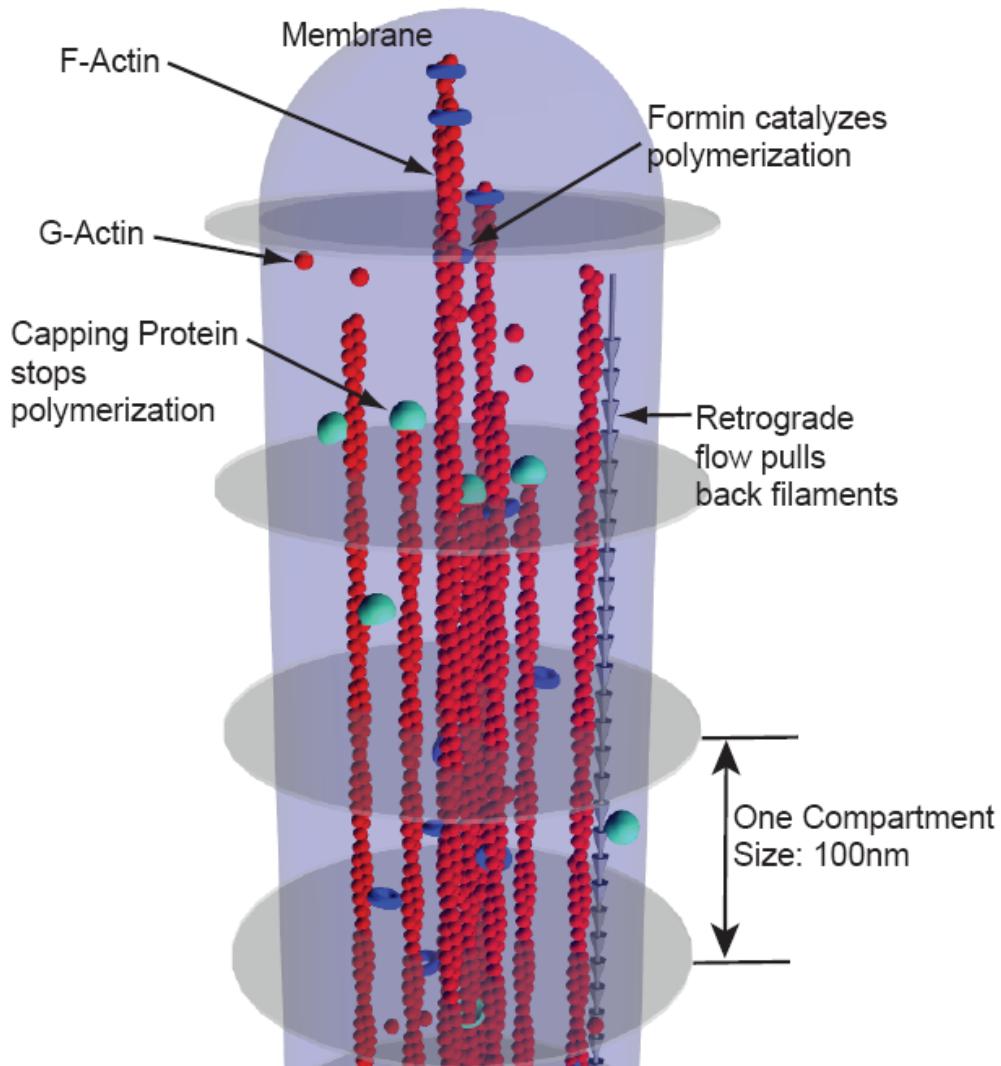
- Stochastic model for both the polymerization and diffusion
- The filopodial tube is partitioned into 50 nm compartments
- G-actin molecules "hop" between neighboring compartments
- Uneven loading of the membrane force among the filaments in the bundle
- Retrograde flow pulls back the actin filaments from the filopodial tube to the cell's body

What limits the length?

F-Actin

Membrane

Formin catalyzes polymerization

G-Actin

Capping Protein stops polymerization

Retrograde flow pulls back filaments

One Compartment Size: 100nm

Lan and Papoian (2008)

$$J_d = -D \frac{\partial c}{\partial z}; \quad \frac{\partial c}{\partial t} + \frac{\partial J_d}{\partial z} = 0;$$

$$J_r = J_p = J_d$$

$$N v_r / \delta = N(k^+ c_{\text{tip}} - k^-) = D(c_{\text{tip}} - c_{\text{base}})/L$$

Y. Lan and G. A. Papoian. The stochastic dynamics of filopodial growth. Biophys J (2008) vol. 94 (10) pp. 3839-3852

Retrograde Flow

Mean-Field Solution

$$h = \frac{C_d l_d}{N}\left(\frac{c_0 \delta}{v_{\text{retr}}}\left(1 - \frac{k_d}{k_n}\right) - \frac{1}{k_0}e^{f\delta/Nk_B T}\right)$$

Stochastic Simulations

Filopodial Length (nm)

$v_{retr}(nm/s)$
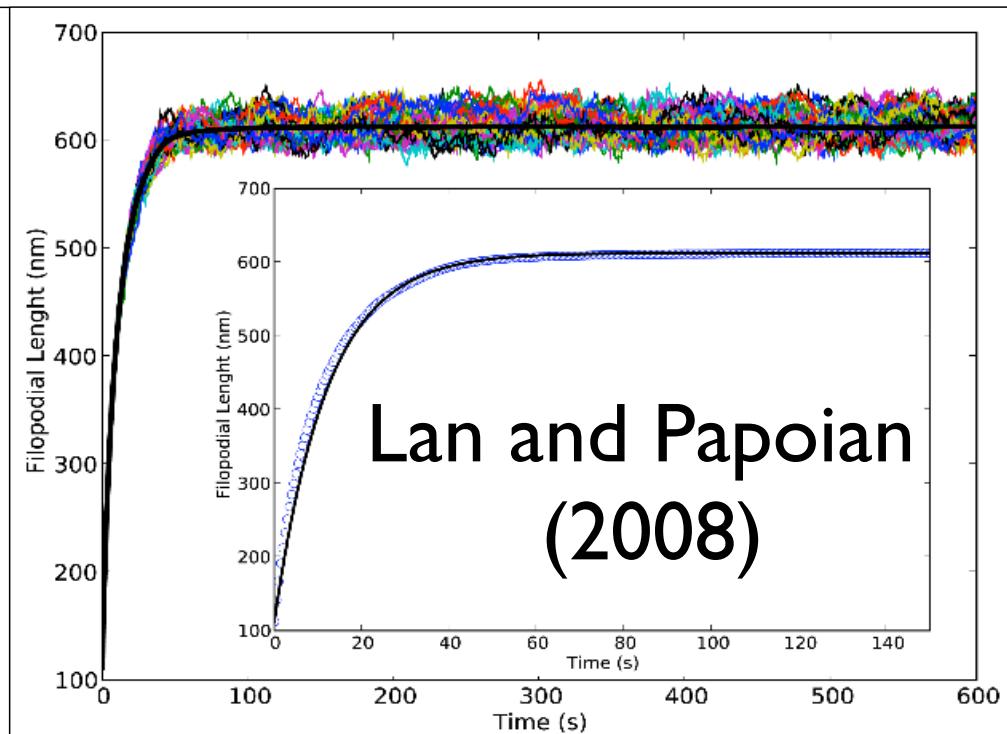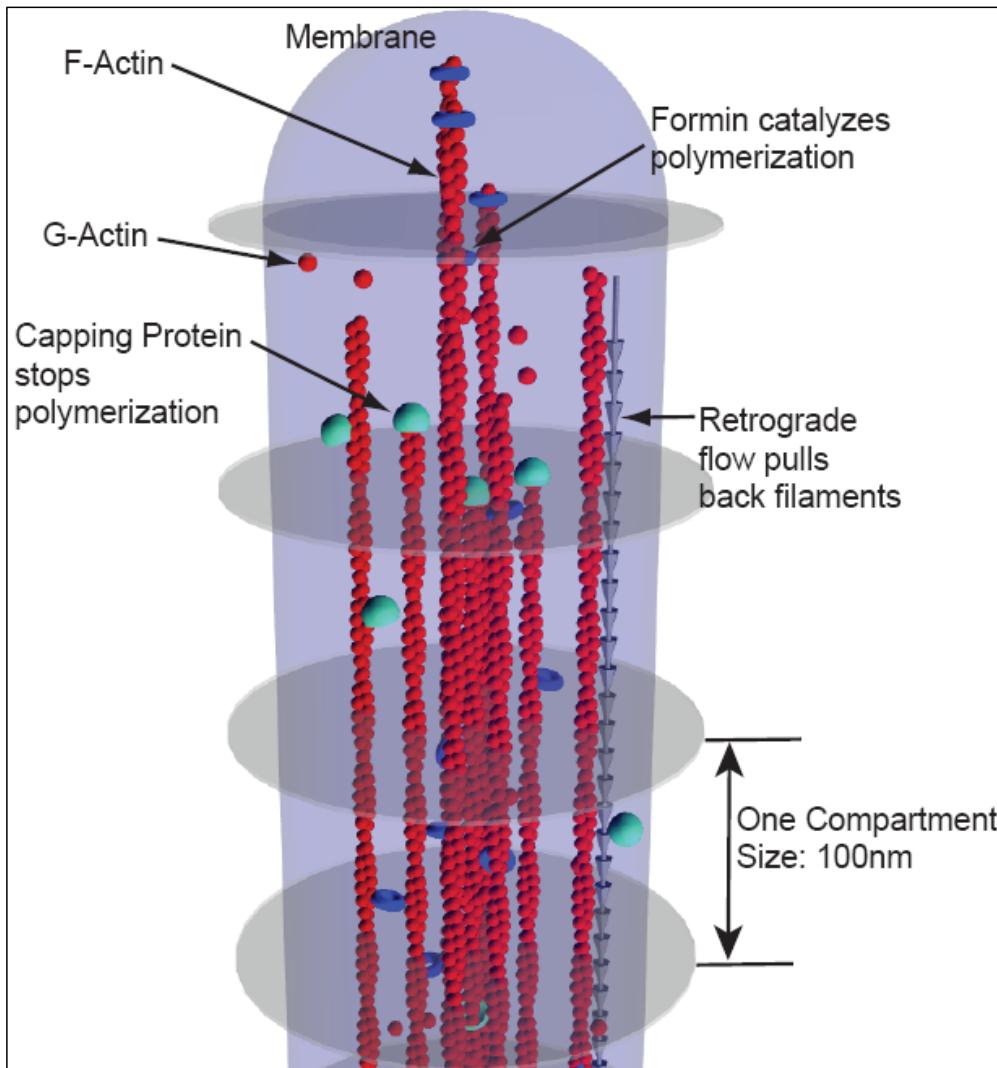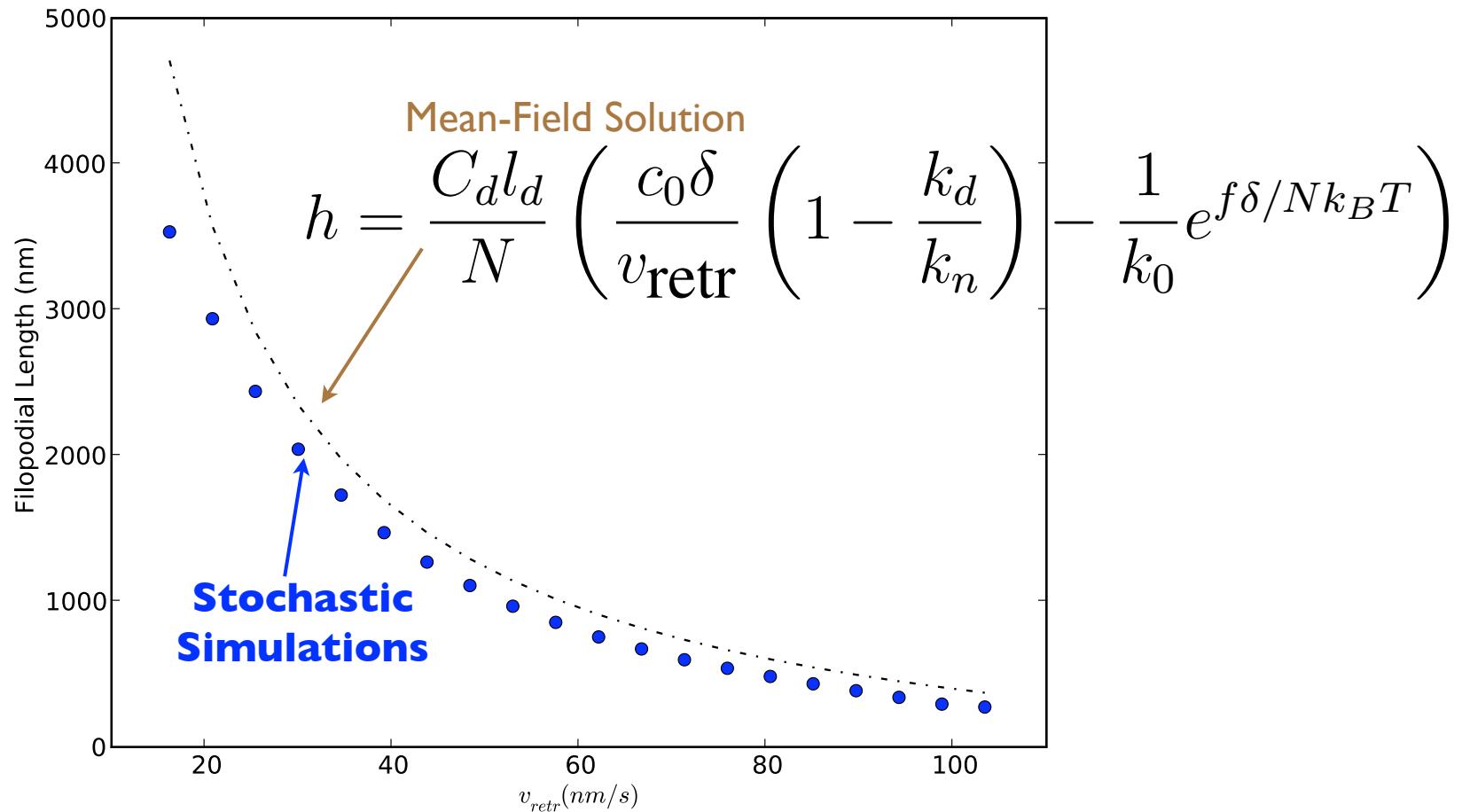
- Filopodia can grow very long at low retrograde flow speeds
- The discrepancy between the mean-field and stochastic solutions can be quite large at low retrograde flow rates

✐ Y. Lan and G. A. Papoian, **Biophys. J.**, (2008) 94, 3839-3852

Adding Capping Proteins & Formins
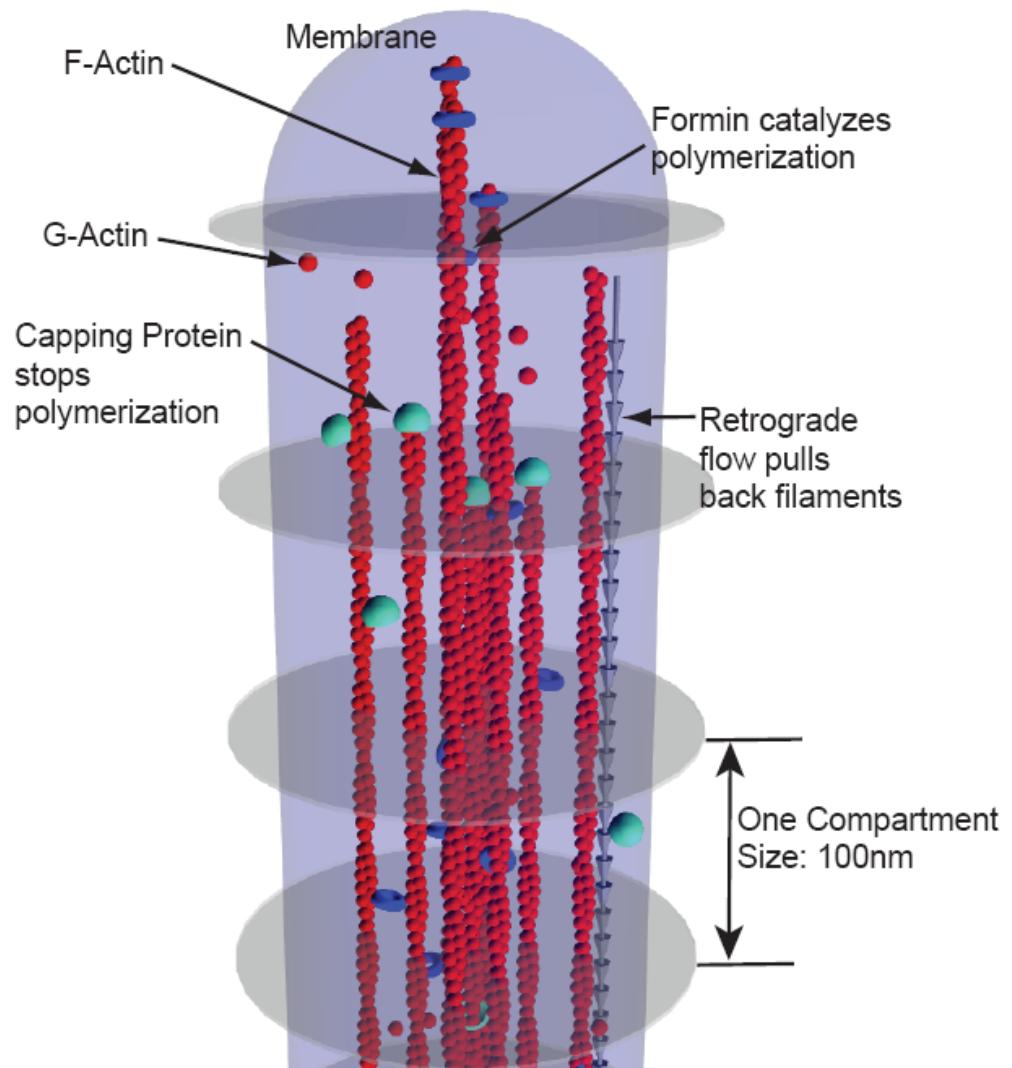
- Capping proteins, when bound to filament tips, arrest polymerization
  - Consequently, the filament starts to retract
  - It may even completely disappear
- Formins accelerate polymerization

✏ P. I. Zhuravlev and G. A. Papoian, **Proc. Natl. Acad. Sci. USA**; *2009*, 106, 11570-11575

Macroscopic oscillations of filapodial length
induced by low concentration of a capping protein
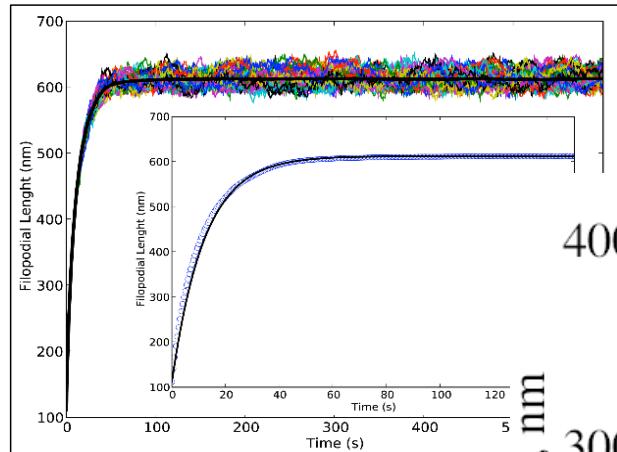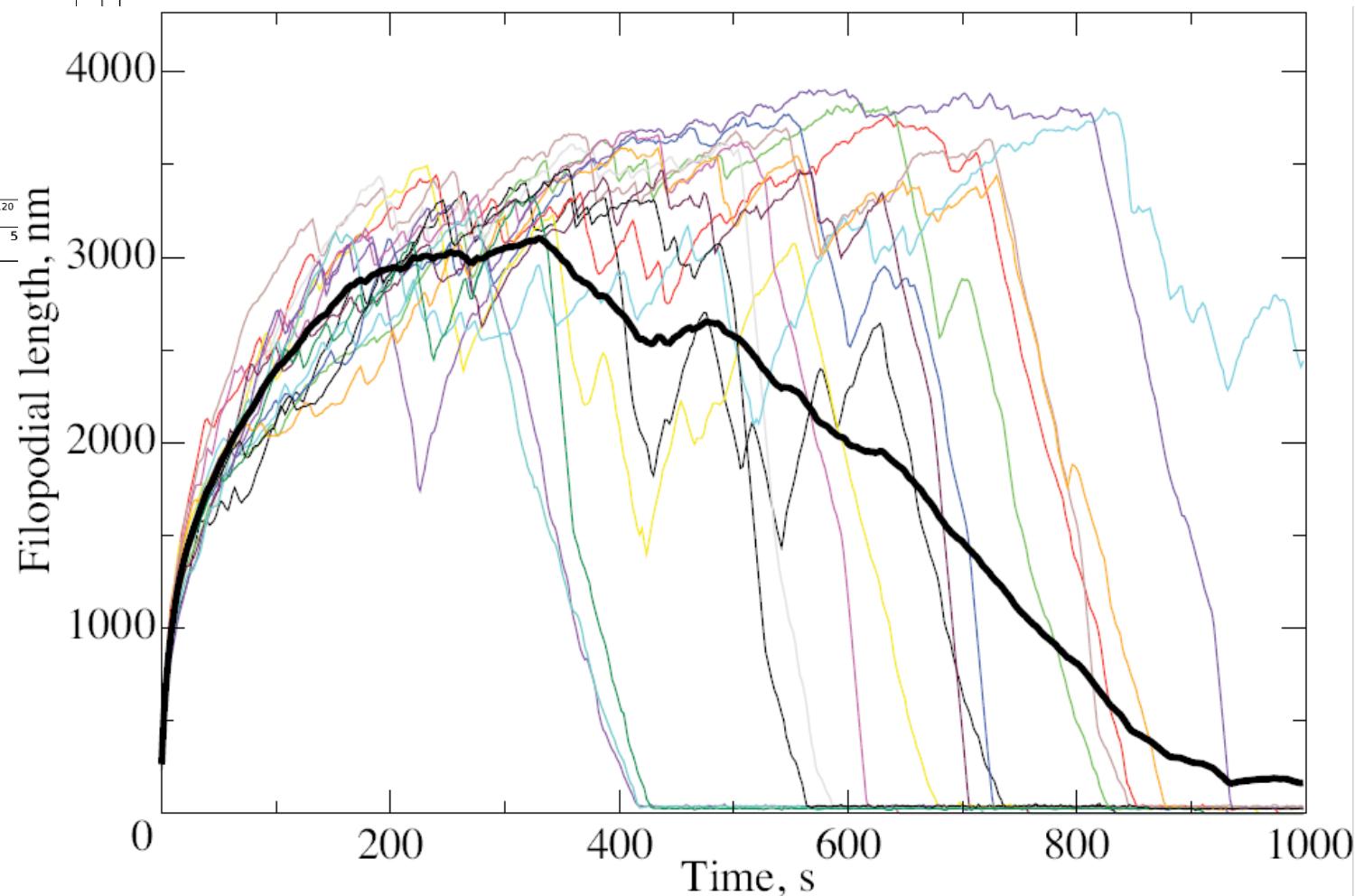
Filopodial Lifetime Distribution
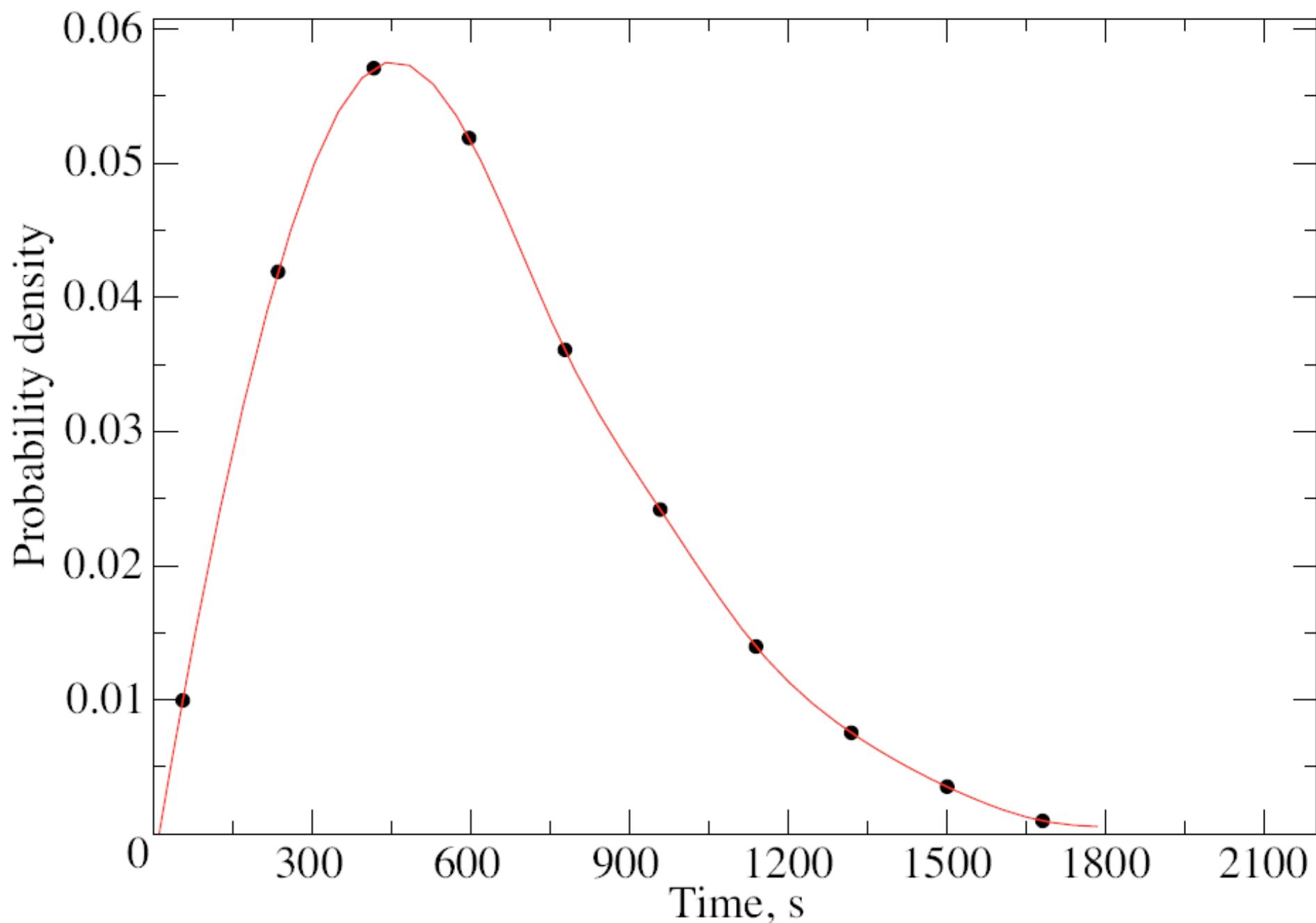
The filopodial length

- Modeling:

  - 0.5 - 2 μm length,

  - growth speed on μm/min scale

  - stationary

- Experiment:

  - typically 1 - 10 μm,

  - up to 100 μm with 10 μm/min growth speed

  - growth - retraction cycles

What limits the length?

Membrane

F-Actin

Formin catalyzes polymerization

G-Actin

Capping Protein stops polymerization

Retrograde flow pulls back filaments

One Compartment Size: 100nm

Lan and Papoian (2008)

$$J_d = -D \frac{\partial c}{\partial z}; \quad \frac{\partial c}{\partial t} + \frac{\partial J_d}{\partial z} = 0;$$

$$J_r = J_p = J_d$$

$$N v_{\mathrm{r}}/\delta = N(k^+ c_{\mathrm{tip}} - k^-) = D(c_{\mathrm{tip}} - c_{\mathrm{base}})/L$$

Y. Lan and G. A. Papoian. The stochastic dynamics of filopodial growth. Biophys J (2008) vol. 94 (10) pp. 3839-3852
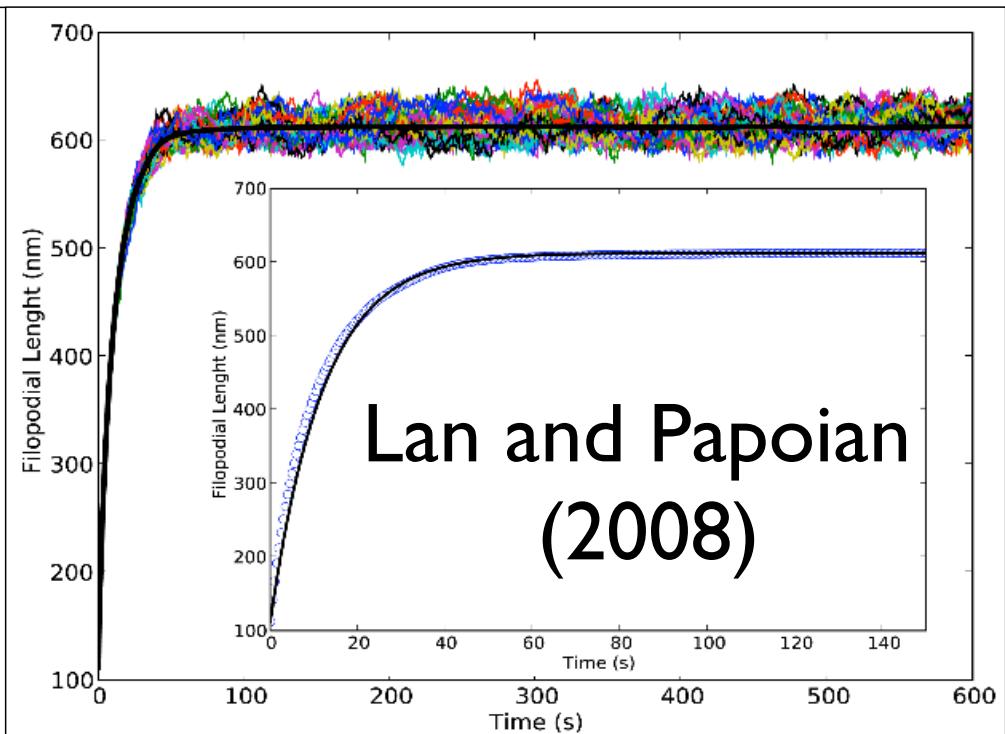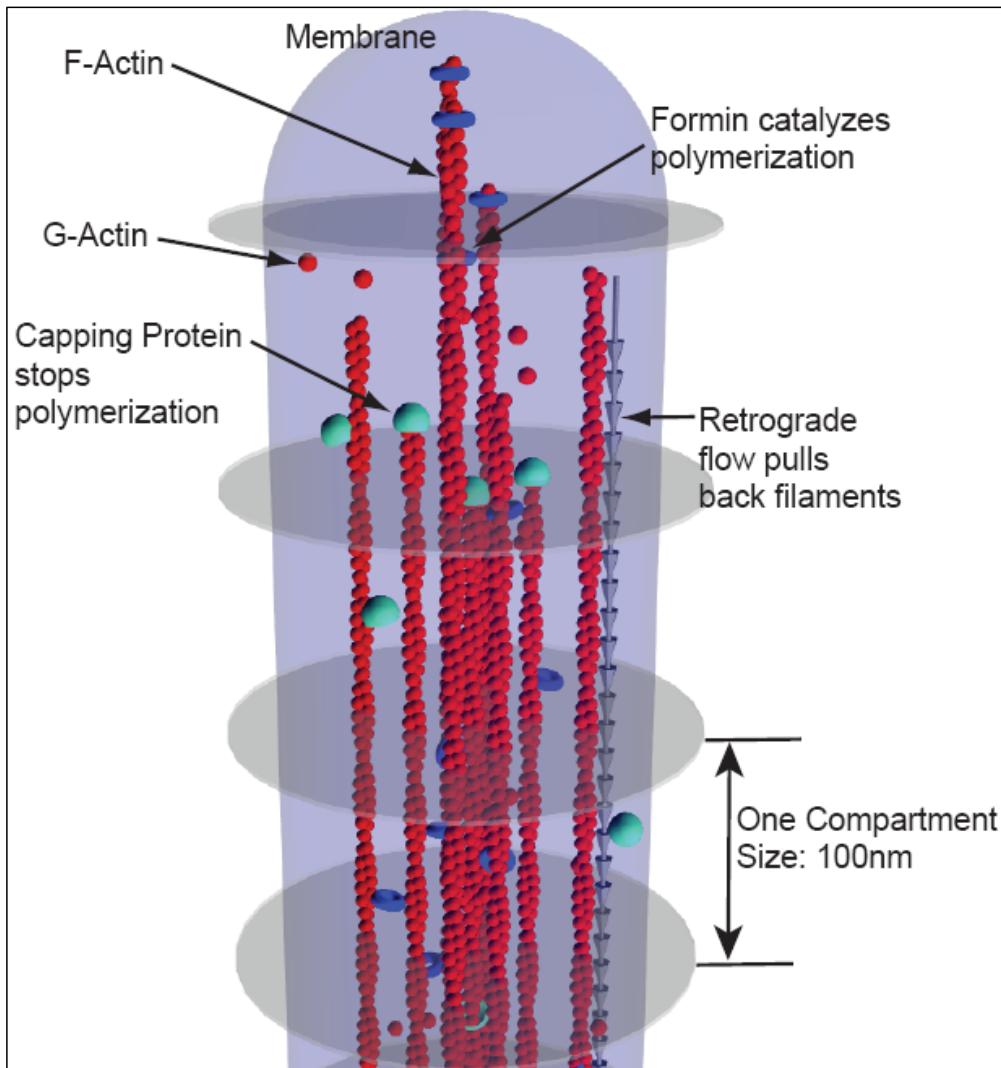
Myosin X

Zhuravlev, Der and Papoian (2010):

Myosin X transports G-actin?
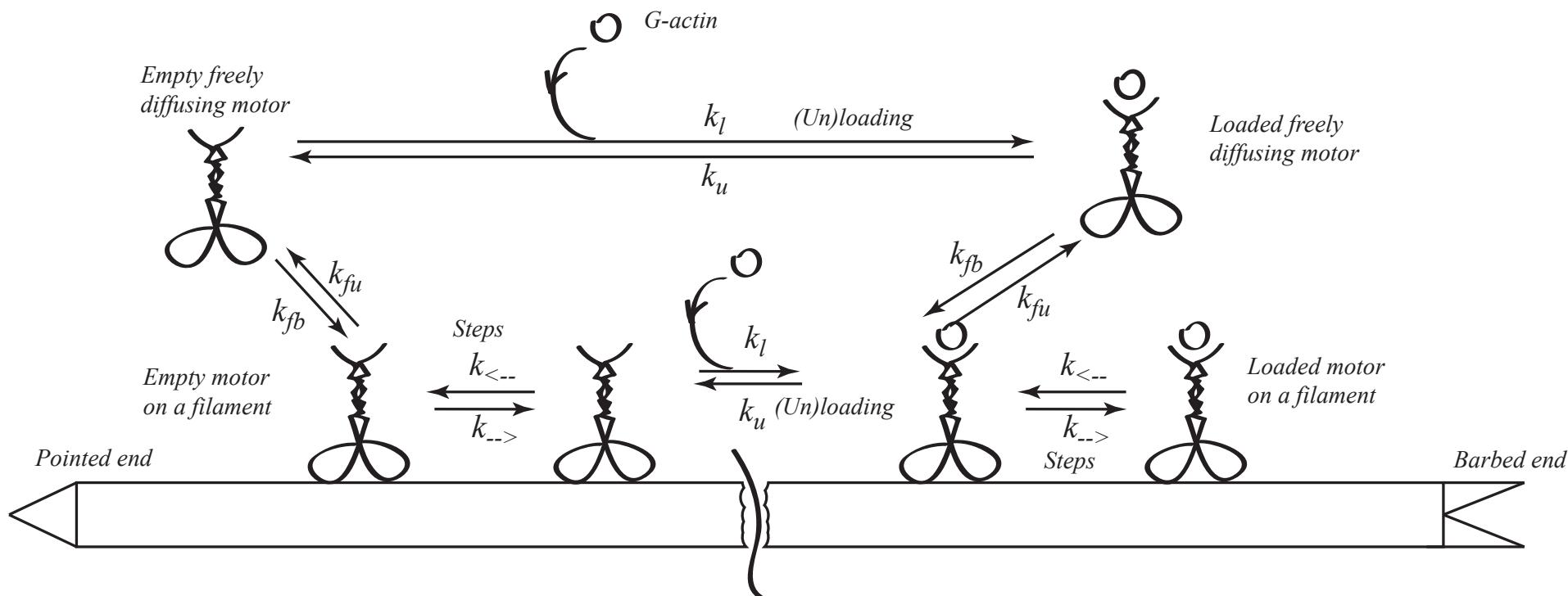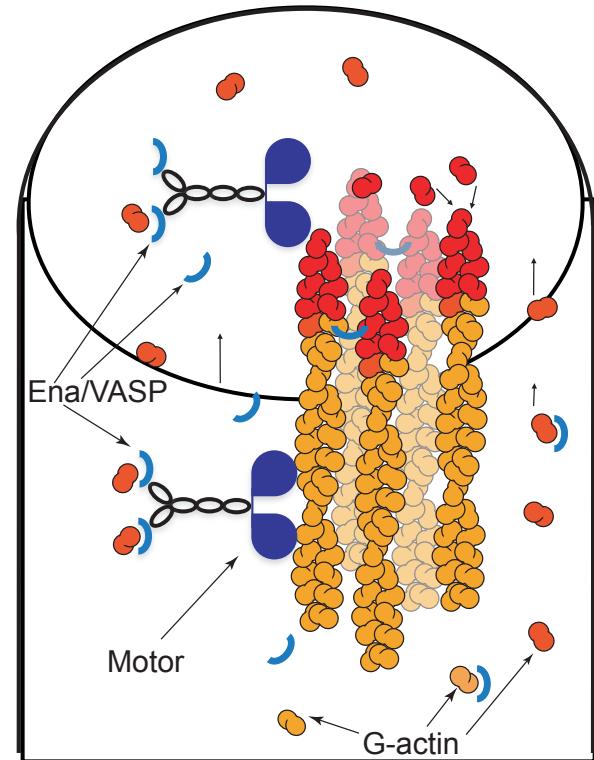
Effect of active transport on filopodial length
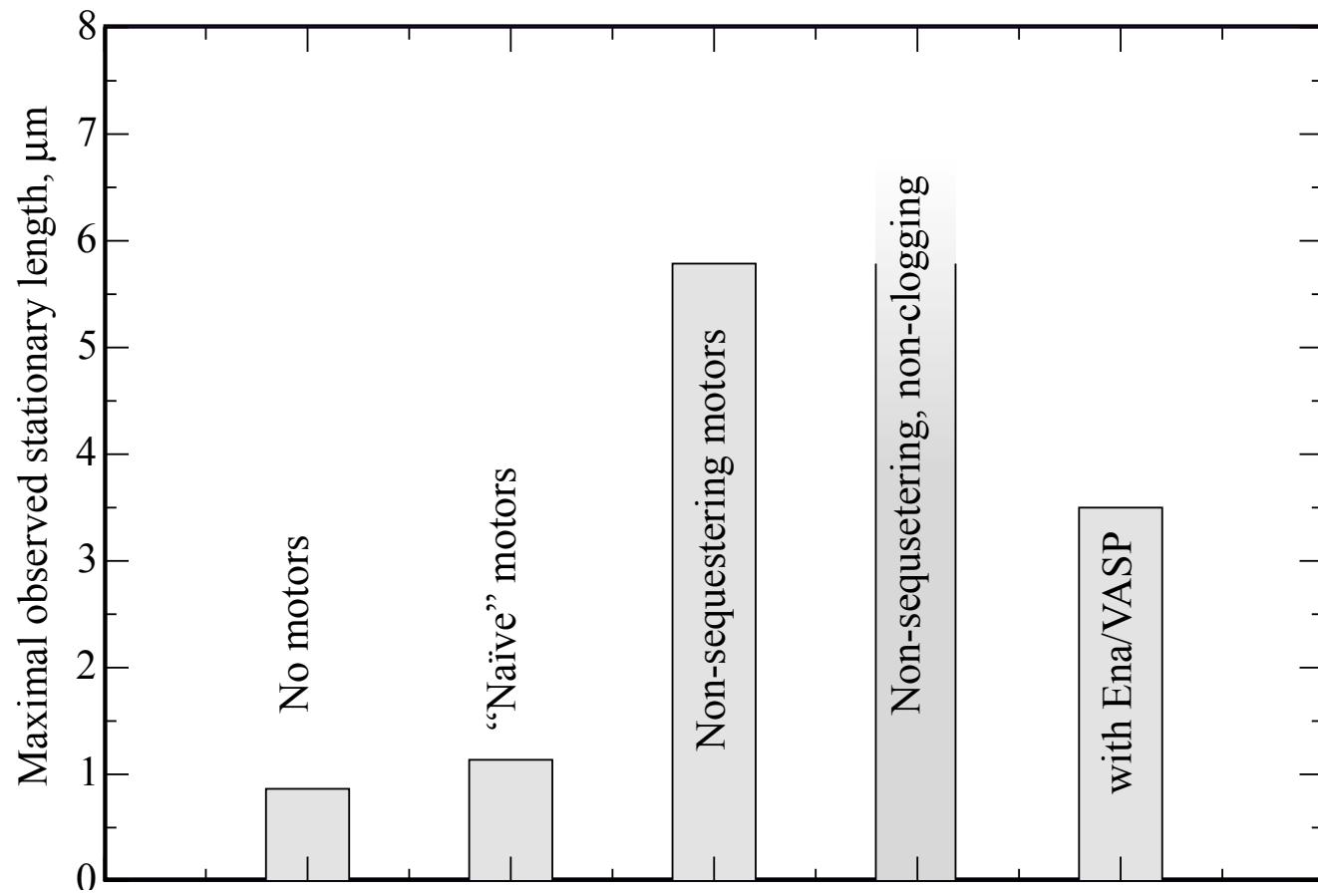
Active Transport Conclusions

- Naïve addition of motors does not work

- There are rules for efficient active transport:

    - Prevent sequestration

    - Clear the "rails"

- Ena/VASP scheme may be a way to achieve these requirements

- Flux balance dramatically affects growth dynamics

Motor concentration profiles
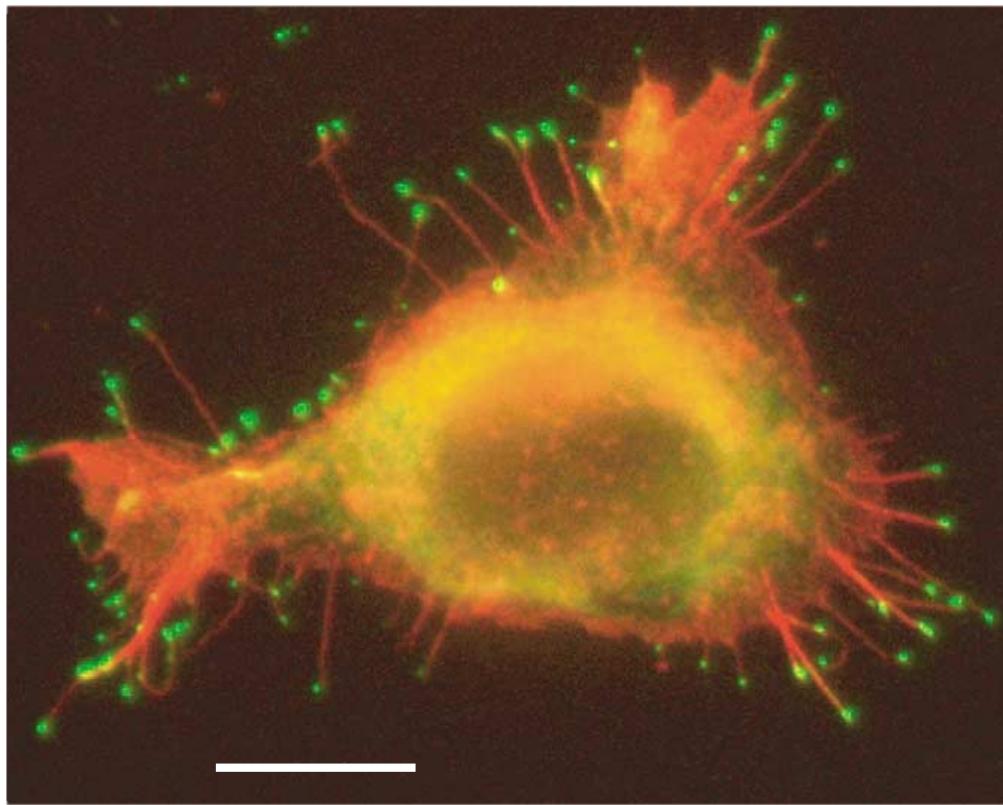
Sousa and Cheney (2005)

Kerber et al. (2009)

Motor Distributions
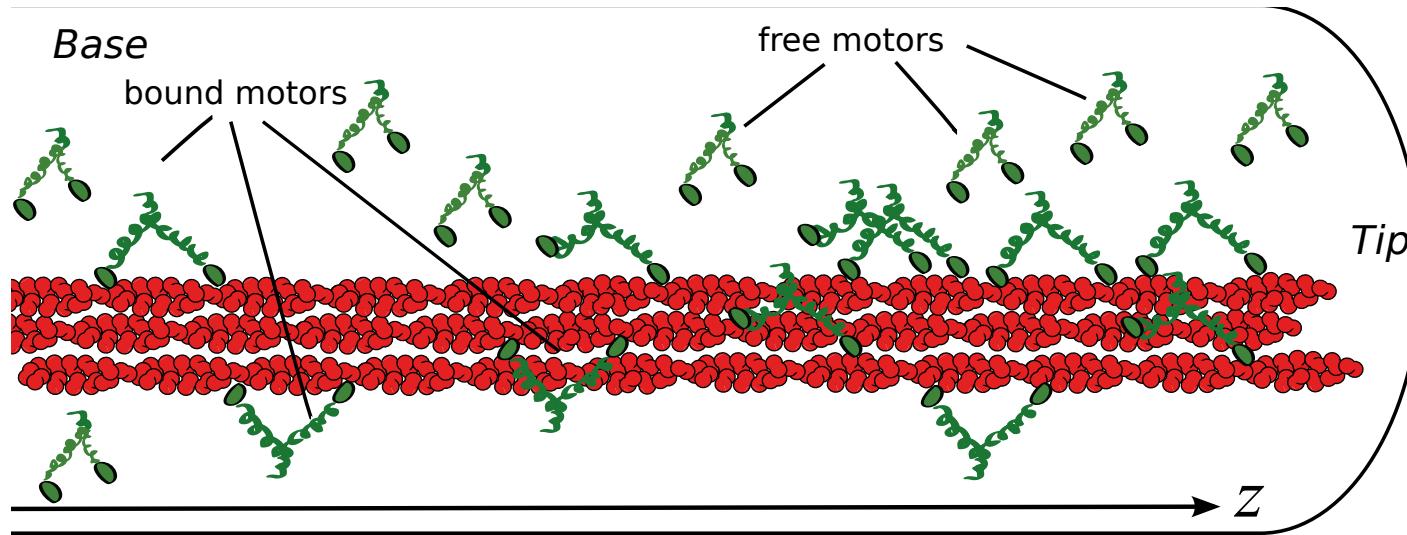
TASEP + diffusion:

$$\frac{\partial c_{\mathrm{f}}}{\partial t} + \frac{\partial J_{\mathrm{f}}}{\partial z} = k_{\mathrm{off}} c_{\mathrm{b}} - k_{\mathrm{on}} c_{\mathrm{f}}$$

$$\frac{\partial c_{\mathrm{b}}}{\partial t} + \frac{\partial J_{\mathrm{b}}}{\partial z} = k_{\mathrm{on}} c_{\mathrm{f}} - k_{\mathrm{off}} c_{\mathrm{b}}$$

$$J_{\mathrm{f}}(z) = -D \frac{\partial c_{\mathrm{f}}}{\partial z}$$

Boundary conditions for stationary solution:

$$J_{\mathrm{f}}(0) = c_{\mathrm{f}}(0), \, (J_{\mathrm{f}} + J_{\mathrm{b}}) \Big|_{tip} = 0, \, J_{\mathrm{b}}(0) = 0$$

$J_{\mathrm{f}} + J_{\mathrm{b}}$ is an integral

The solution does not depend on the filopodial length!

Master Equation: Neglecting Correlations Between Sites

- Discrete hopping for **bound** motors

- Continuous diffusion for **free** motors

$$\dot{b}_n = k_{\rightarrow} b_{n-1} + k_{\leftarrow} b_{n+1} - (k_{\rightarrow} + k_{\leftarrow}) b_n - k_{\text{off}} b_n + k_{\text{on}} c_{\text{f}}(z_n)$$

$$\frac{\partial c_{\text{f}}}{\partial t} = \frac{\partial}{\partial z}\left(D_{\text{m}} \frac{\partial c_{\text{f}}}{\partial z}\right) + k_{\text{off}} c_{\text{b}} - k_{\text{on}} c_{\text{f}},$$

$$z_n = n\varepsilon \qquad\qquad v = (k_{\rightarrow} - k_{\leftarrow})/\varepsilon$$
$$b_n = b(z_n) = c_{\text{b}}(z)$$
$$b_{n-1} = c_{\text{b}}(z - \varepsilon) = c_{\text{b}}(z) - \varepsilon c'_{\text{b}}(z) + \dots$$
$$b_{n+1} = c_{\text{b}}(z + \varepsilon) = c_{\text{b}}(z) + \varepsilon c'_{\text{b}}(z) + \dots$$

$$\frac{\partial c_{\text{b}}}{\partial t} = -\frac{\partial}{\partial z}\left(v c_{\text{b}}\right) - k_{\text{off}} c_{\text{b}} + k_{\text{on}} c_{\text{f}}$$

More Accurate Semi-Mean-Field Equation

$$\dot{b}_n = k_{\rightarrow} b_{n-1}(1 - b_n) + k_{\leftarrow} b_{n+1}(1 - b_n) - k_{\rightarrow}(1 - b_{n+1})b_n -$$
$$- k_{\leftarrow}(1 - b_{n-1})b_n - k_{\mathrm{off}}b_n + k_{\mathrm{on}}(1 - b_n)c_{\mathrm{f}}(z_n)$$

$$\dot{b}_n = k_{\rightarrow} b_{n-1} + k_{\leftarrow} b_{n+1} - (k_{\rightarrow} + k_{\leftarrow})b_n -$$
$$- b_n(k_{\rightarrow} - k_{\leftarrow})(b_{n-1} - b_{n+1}) - k_{\mathrm{off}}b_n + k_{\mathrm{on}}(1 - b_n)c_{\mathrm{f}}(z_n)$$

$$\frac{\partial c_{\mathrm{b}}}{\partial t} = -\frac{\partial}{\partial z}\left(v c_{\mathrm{b}}\right) + 2 v c_{\mathrm{b}}\frac{\partial c_{\mathrm{b}}}{\partial z} - k_{\mathrm{off}} c_{\mathrm{b}} + k_{\mathrm{on}}(1 - c_{\mathrm{b}})c_{\mathrm{f}}$$

$$\frac{\partial c_{\mathrm{b}}}{\partial t} = -\frac{\partial}{\partial z}\left(v c_{\mathrm{b}}(1 - c_{\mathrm{b}})\right) - k_{\mathrm{off}} c_{\mathrm{b}} + k_{\mathrm{on}}(1 - c_{\mathrm{b}})c_{\mathrm{f}}$$

...and then we start neglecting terms

Phantom Motors : Neglecting Next Neighbor Correlations

Naoz et al. , Biophys J, 2008 (stereocilia):

$$\begin{cases} -D_{\mathrm{m}}\frac{\partial^2 c_{\mathrm{f}}}{\partial z^2} = k_{\mathrm{off}}c_{\mathrm{b}} - k_{\mathrm{on}}c_{\mathrm{f}}, \\ v\frac{\partial c_{\mathrm{b}}}{\partial z} = -k_{\mathrm{off}}c_{\mathrm{b}} + k_{\mathrm{on}}c_{\mathrm{f}}, \end{cases}$$

- Analytical exponential solution blows up near the tube base

Plot axes: $c_{\mathrm{b}}, c_{\mathrm{f}}$, μM versus z (along the filopodium from the base), nm

Inset axis: c_{f}, μM

Legend:
- Phantom motors (dashed)
- Finite filament capacity (solid)
- Simulations (■ ●)
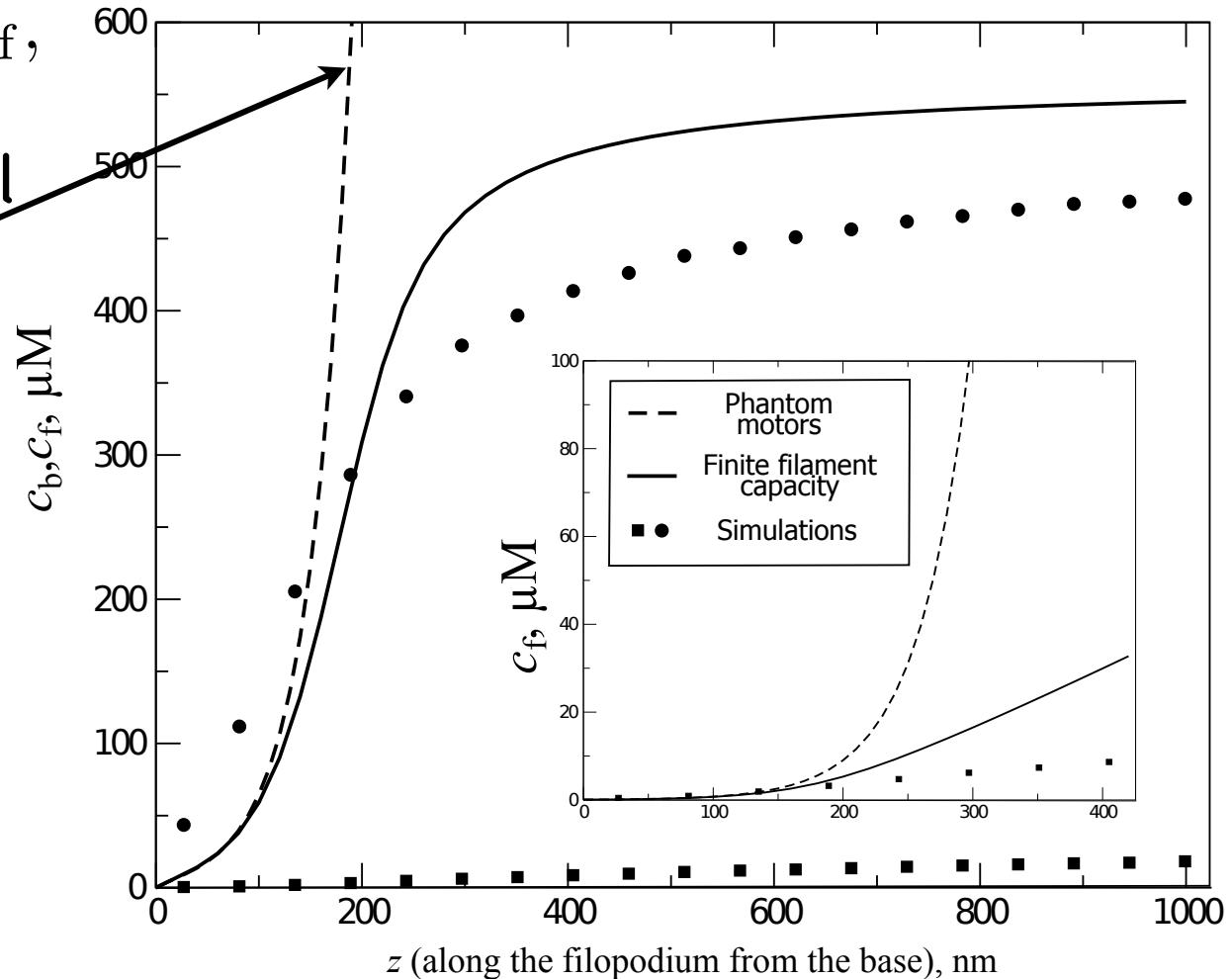
Finite Filament Capacity: Saturation Effect

$$\begin{cases} -D_{\mathrm{m}} \frac{\partial^2 c_{\mathrm{f}}}{\partial z^2} = k_{\mathrm{off}} c_{\mathrm{b}} - k_{\mathrm{on}} \left(1 - c_{\mathrm{b}}\right) c_{\mathrm{f}}, \\ v \frac{\partial c_{\mathrm{b}}}{\partial z} = -k_{\mathrm{off}} c_{\mathrm{b}} + k_{\mathrm{on}} \left(1 - c_{\mathrm{b}}\right) c_{\mathrm{f}}, \end{cases}$$

"on" rate diminishes with the fraction of bound filaments:

$$k_{\mathrm{on}}(c_{\mathrm{b}}) = k_{\mathrm{on}}(1 - c_{\mathrm{b}})$$
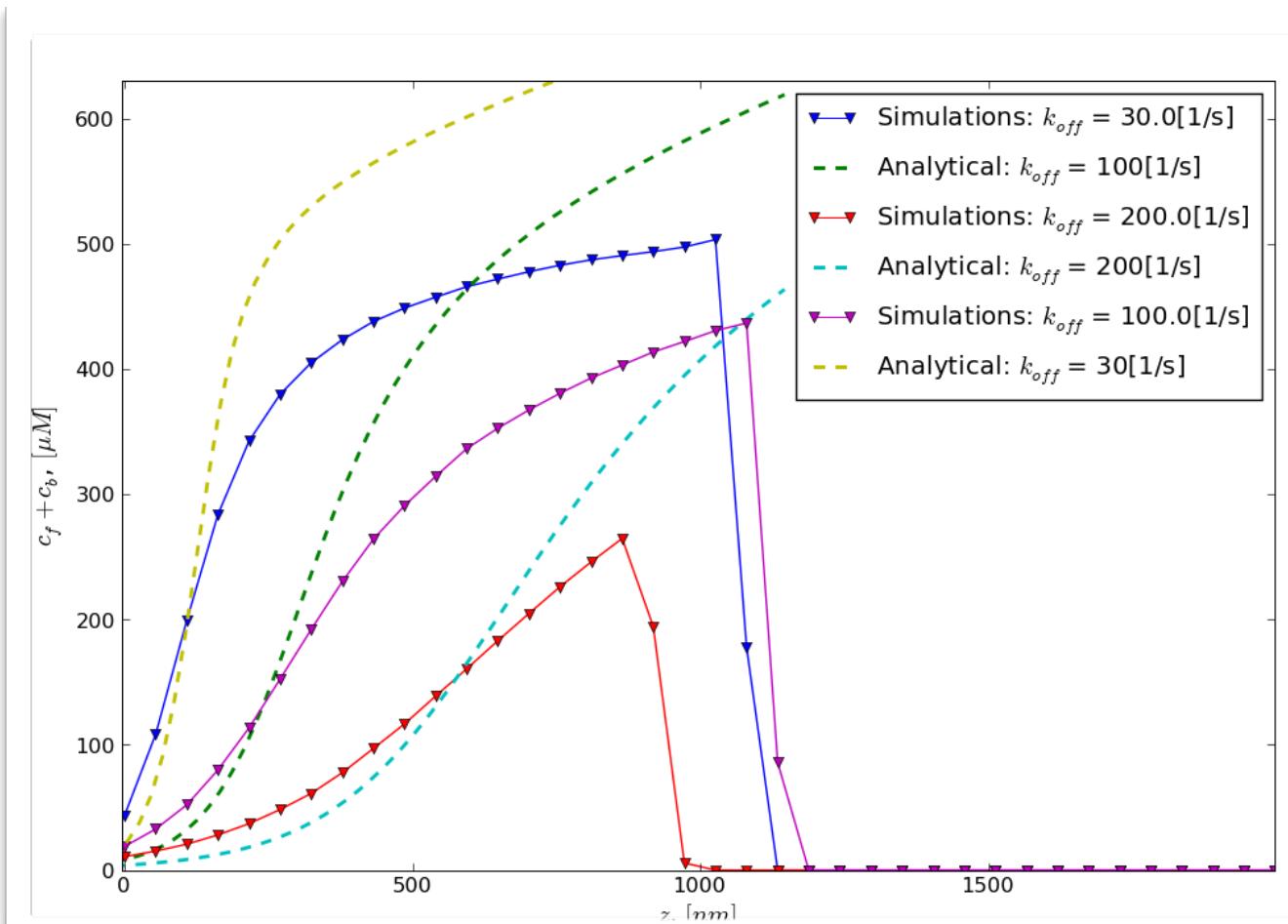
Jammed Motor Model:
Traffic Jam + Saturation Effect

$$\begin{cases} -D_{\mathrm{m}} \frac{\partial^2 c_{\mathrm{f}}}{\partial z^2} = k_{\mathrm{off}} c_{\mathrm{b}} - k_{\mathrm{on}}(1 - c_{\mathrm{b}})c_{\mathrm{f}}, \\ \frac{\partial}{\partial z}\left(v c_{\mathrm{b}}(1 - c_{\mathrm{b}})\right) = -k_{\mathrm{off}} c_{\mathrm{b}} + k_{\mathrm{on}}(1 - c_{\mathrm{b}})c_{\mathrm{f}}, \end{cases}$$

motor speed slows down due to neighbors

Legend:
- k_{off}=100 v=50 [M]=0.1
- k_{off}=100 v=50 [M]=0.3
- k_{off}=30 v=50 [M]=0.1
- k_{off}=200 v=50 [M]=0.1
- k_{off}=30 v=50 [M]=1
- k_{off}=30 v=10 [M]=0.1

$c_{\mathrm{b}}, c_{\mathrm{f}} \mu M$

z (along the filopodium from the base), nm

$c_{\mathrm{f}}, \mu M$