Mathematical Cell Biology Graduate Summer Course

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The actin cytoskeleton and cell motility by protrusion

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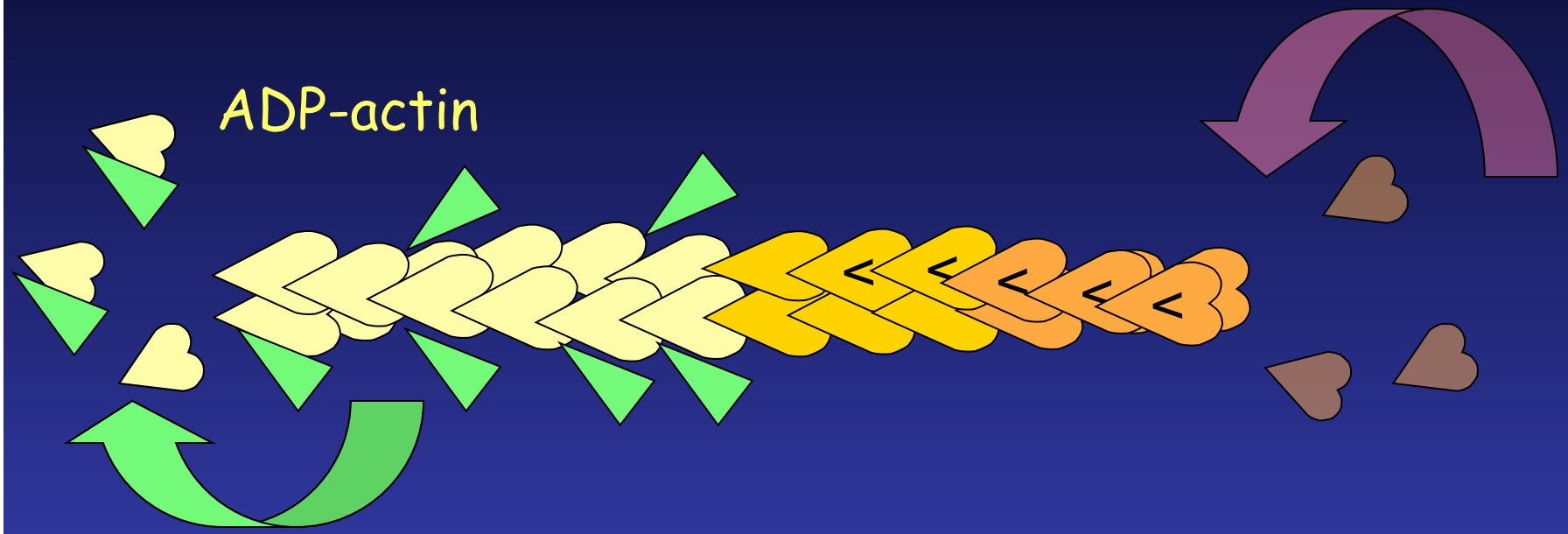
Regulation of Actin Dynamics in Rapidly Moving Cells: A Quantitative Analysis

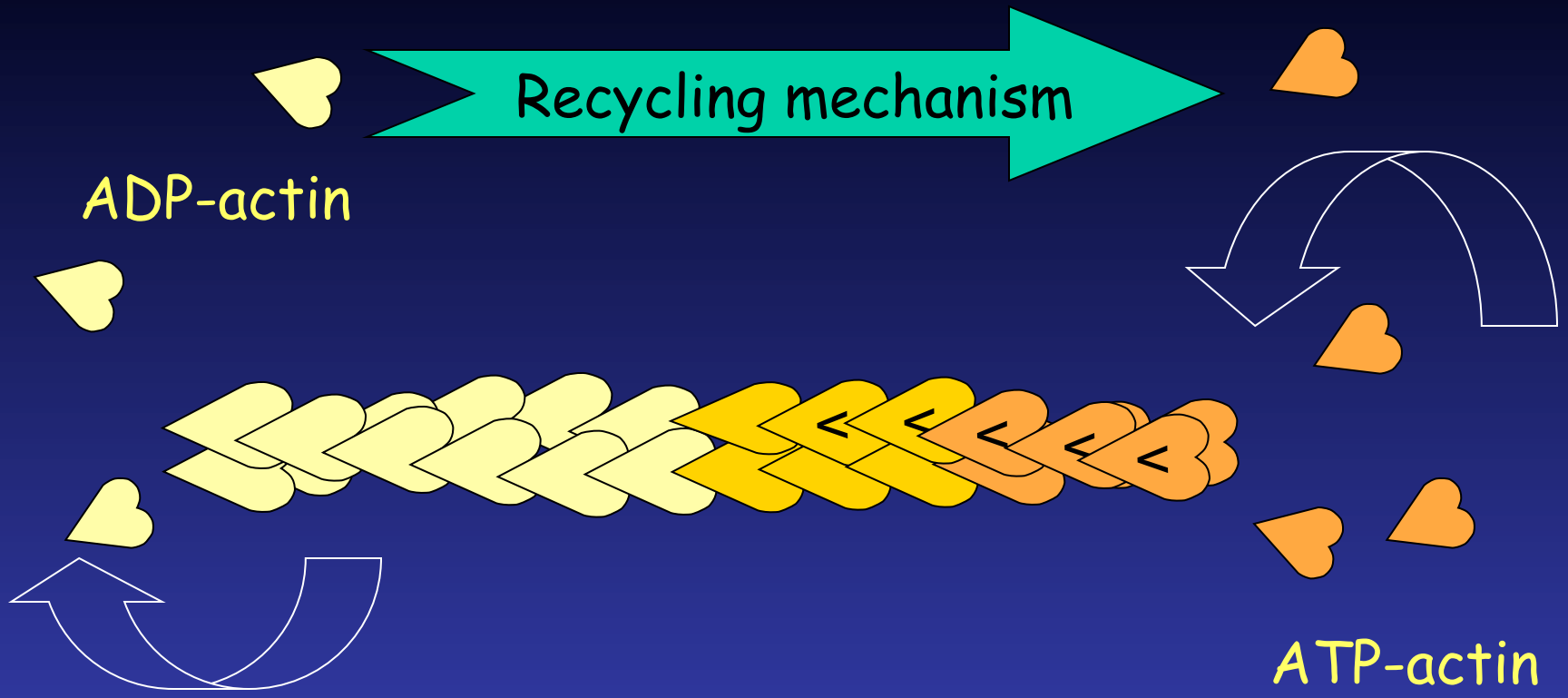
Alex Mogilner* and Leah Edelstein-Keshet†

More advanced actin features

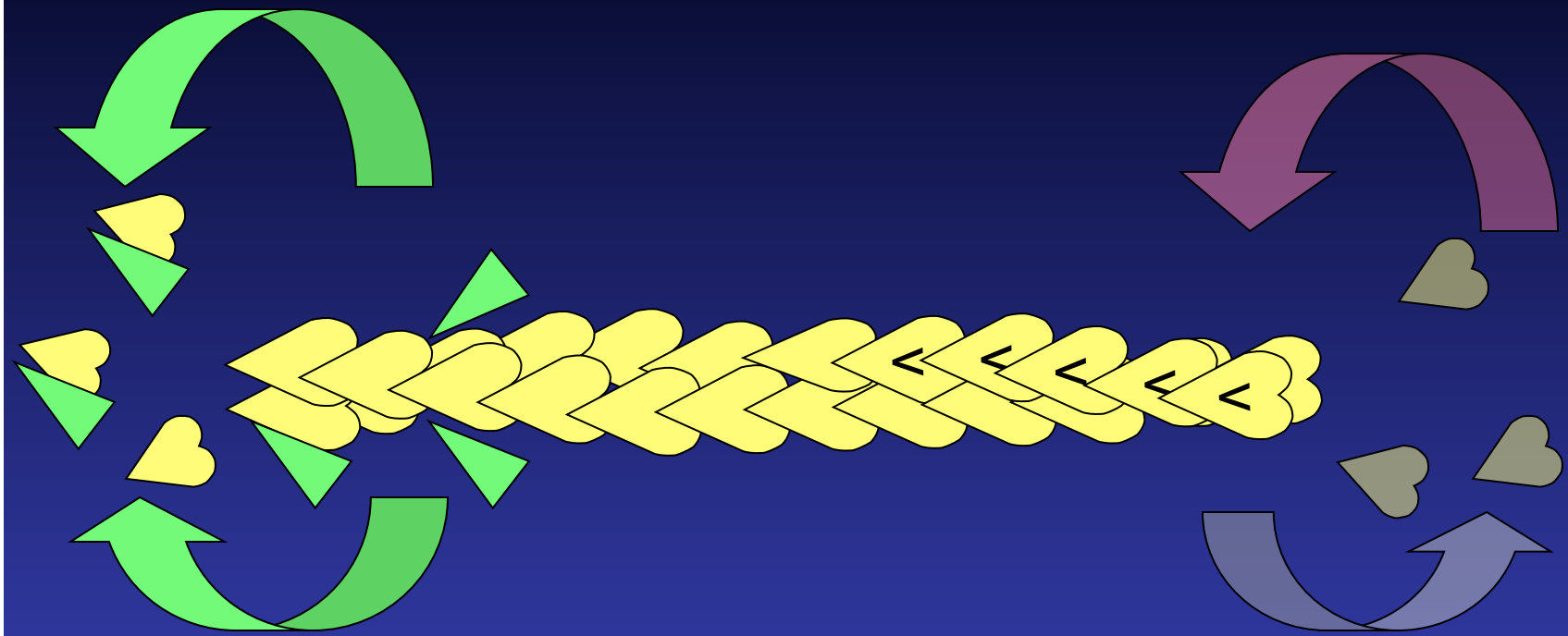
Actin monomers and filaments interact with many other kinds of proteins in the cell.



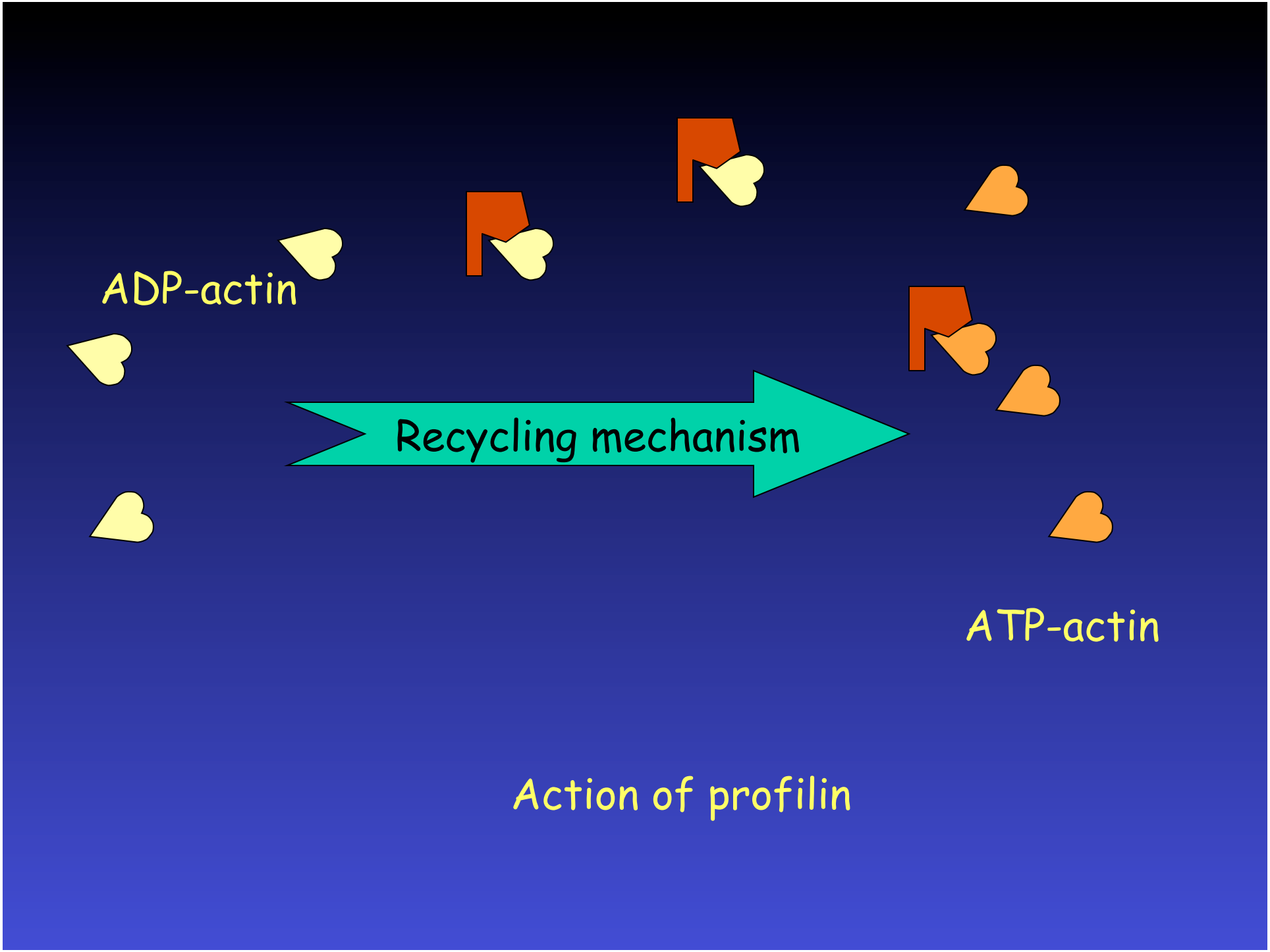
Cutting and fragmenting occurs fastest at the older (ADP-actin) parts of an actin filament



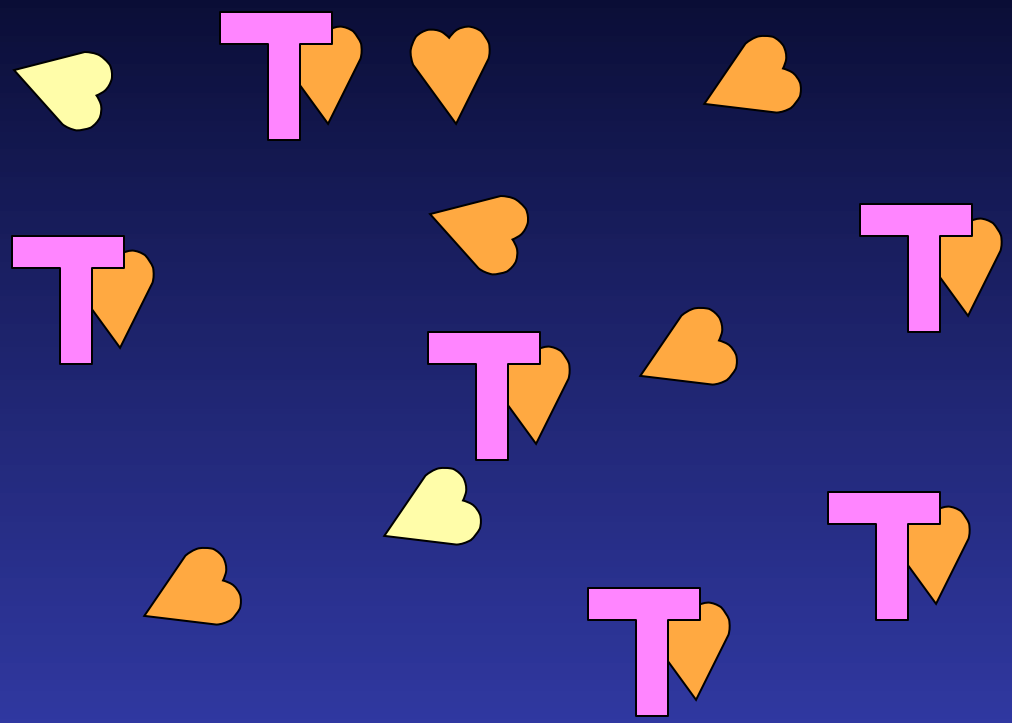
There are mechanisms for converting "spent" ADP-actin monomers into their active form



Agents such as cofilin hasten breakup



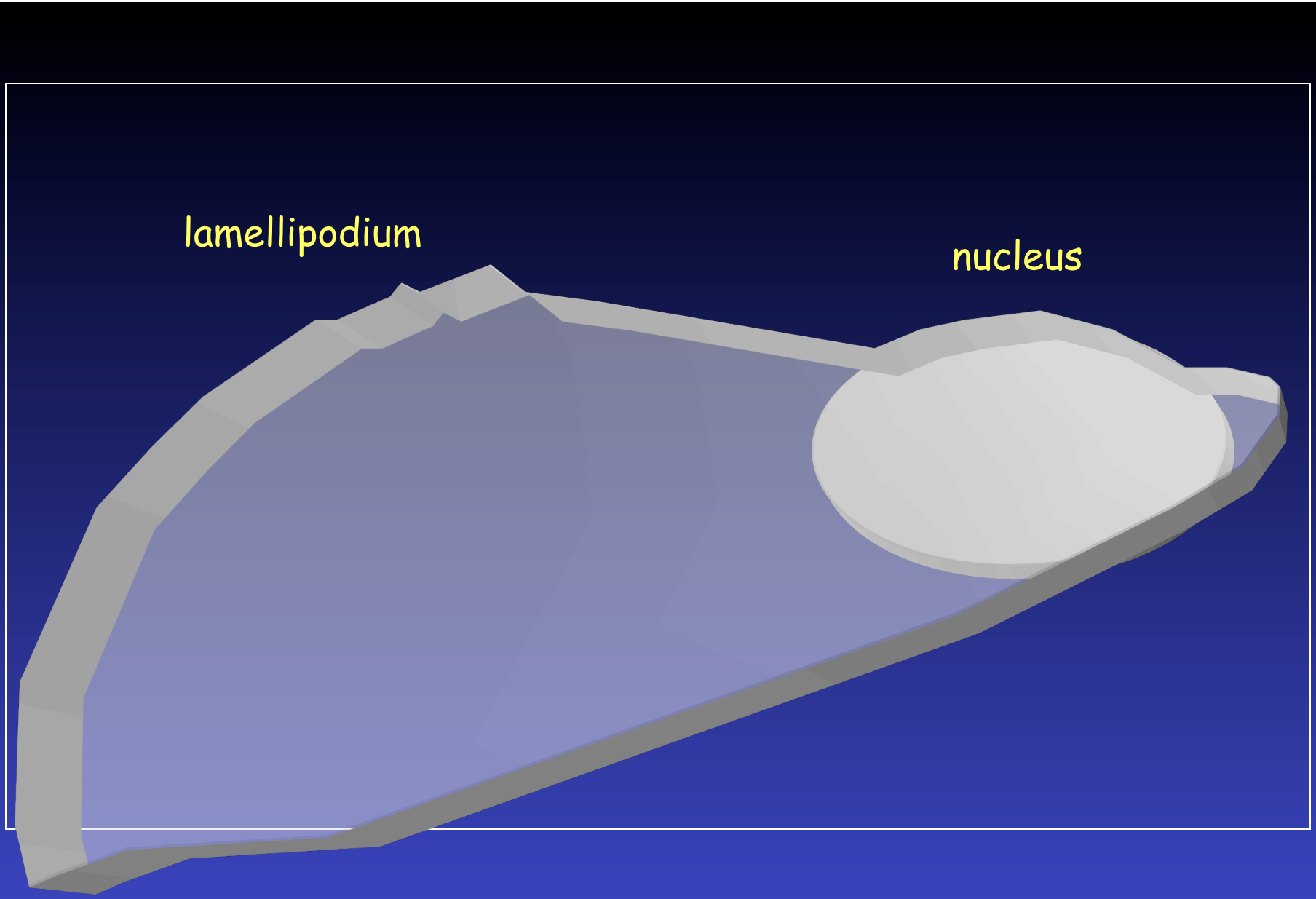
Action of profilin



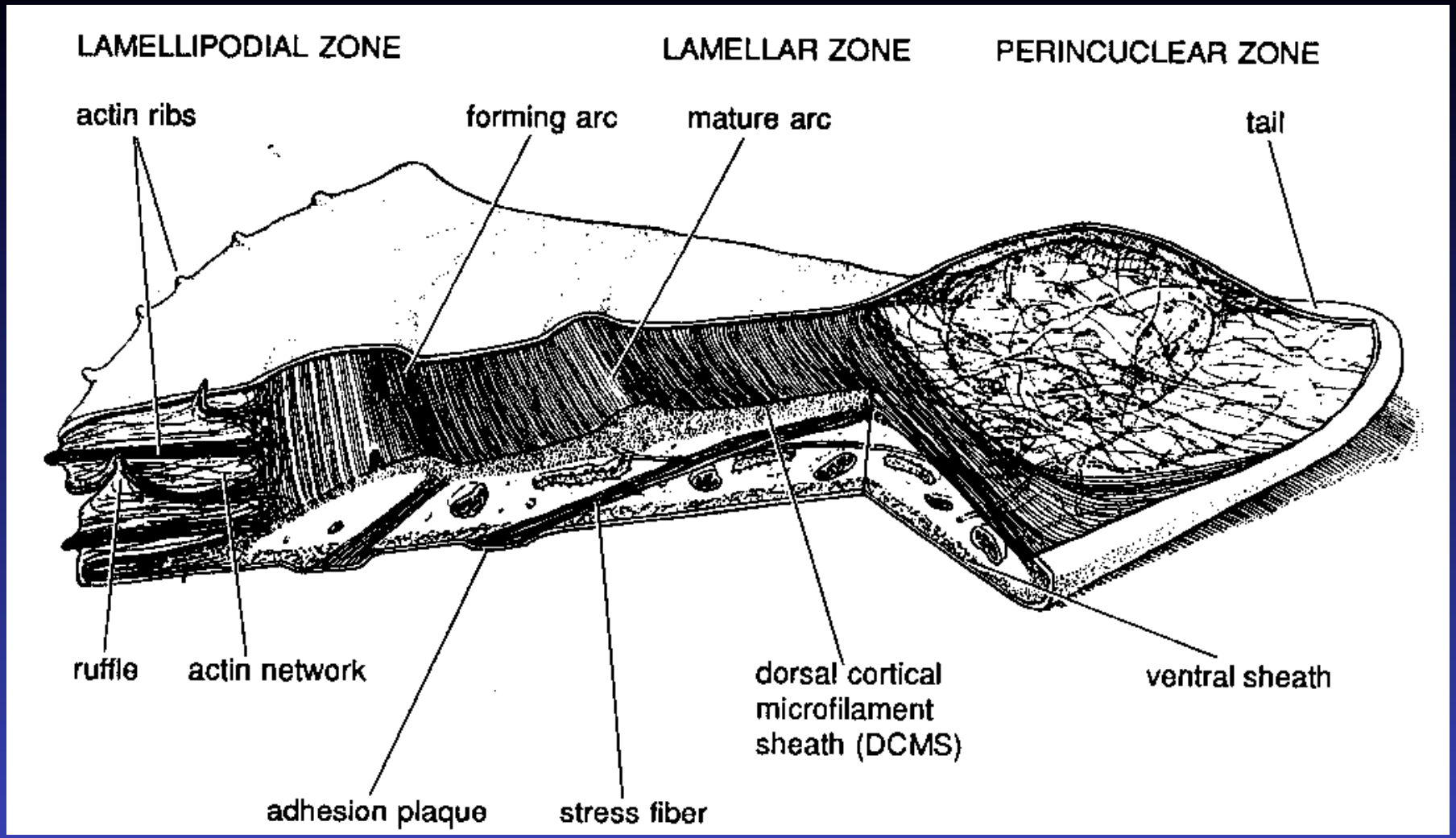
Thymosin sequesters actin monomers

(to control the rate of polymerization)

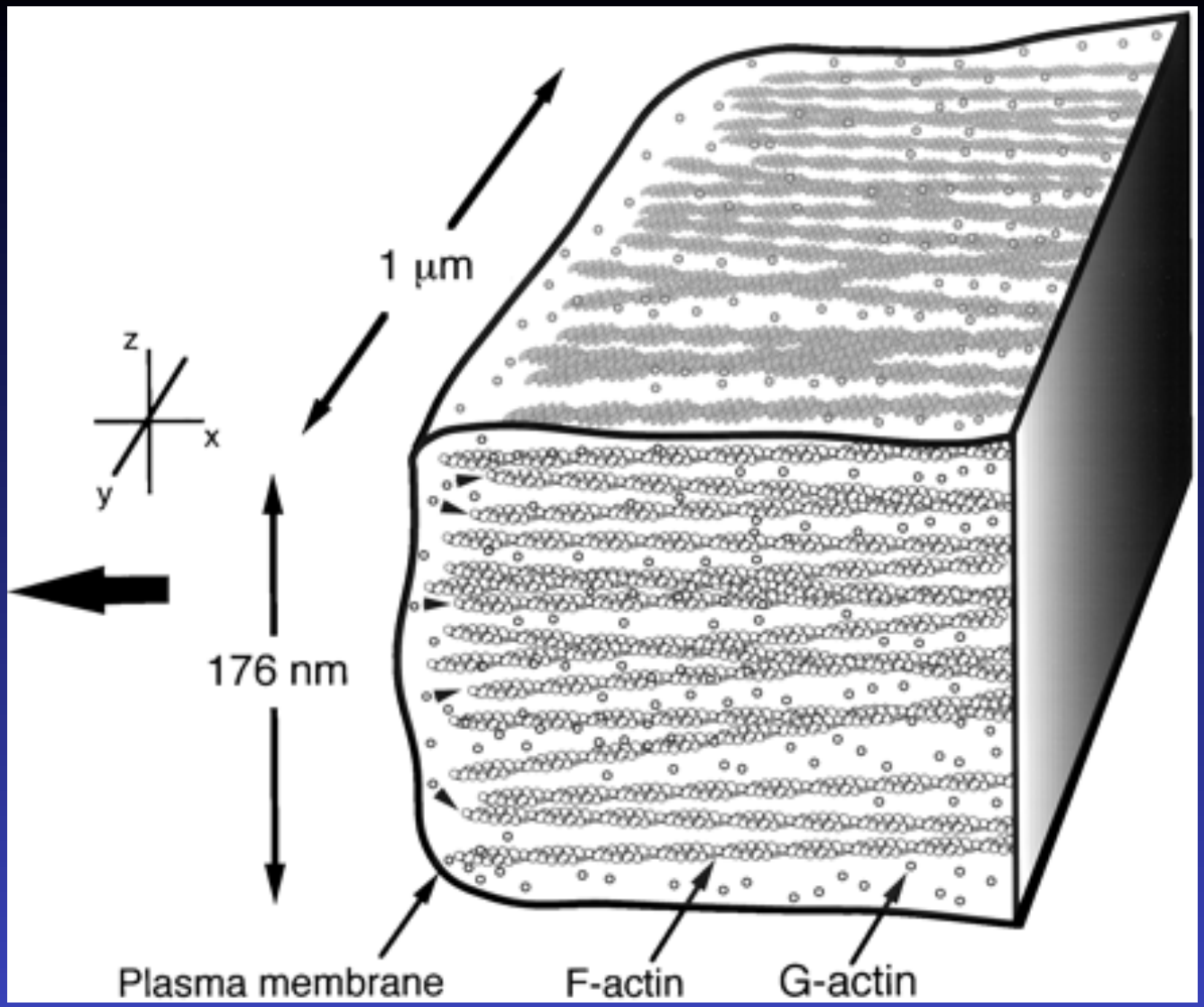
Understanding cell motion requires an in depth understanding of the cytoskeleton, its components and its dynamic properties.



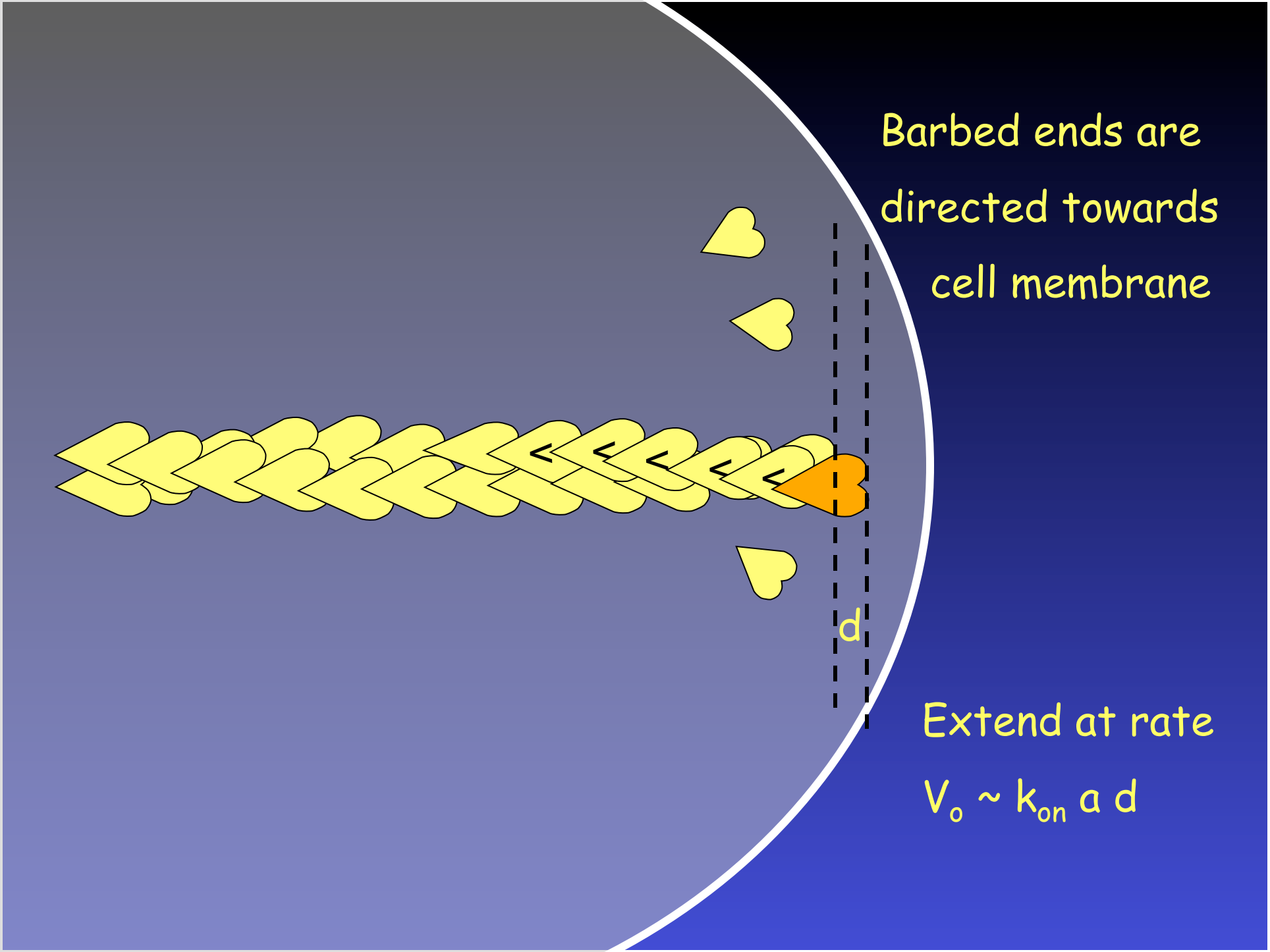
Typical cell shape (fibroblast)

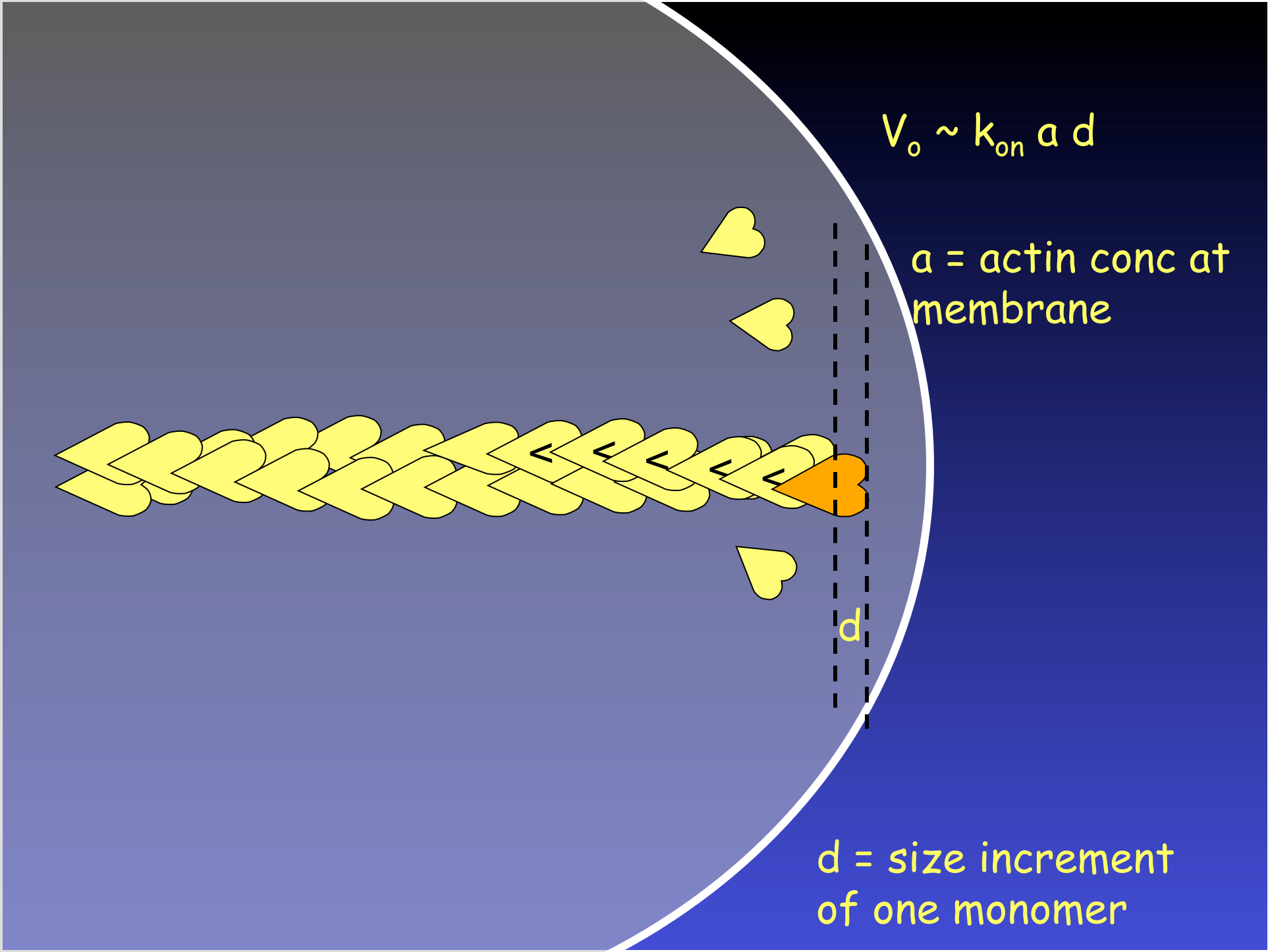


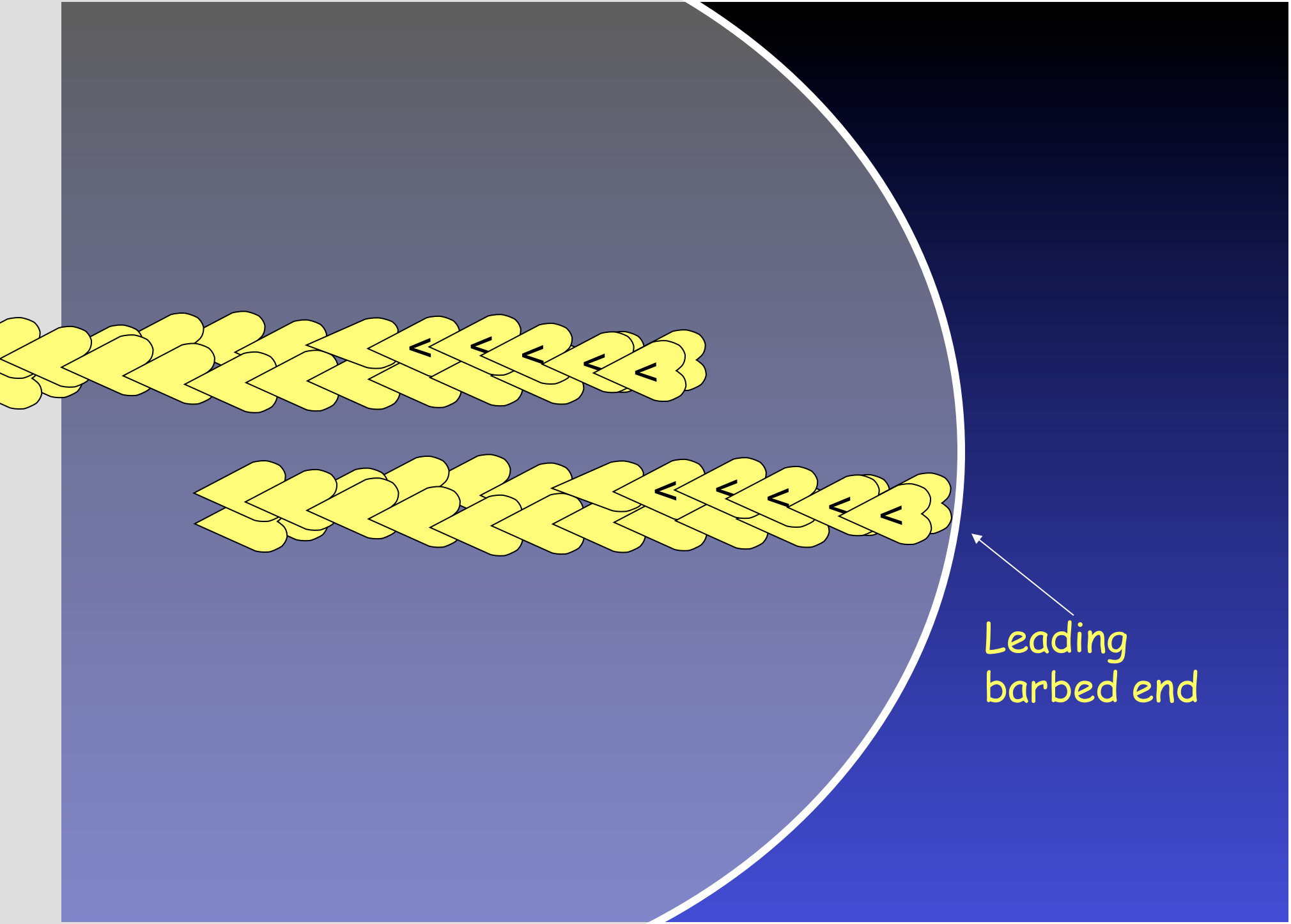
Heath & Holifeld (1993) in: Cell Behaviour, Adhesion, and Motility, Jones, Wigley, Warn, eds Soc Exp Biol Symp 47



Abraham, Krishnamurthi, Taylor, Lanni (1999) Biophys J 77: 1721-1732


Barbed ends are
directed towards
cell membrane
d
Extend at rate
$V_o \sim k_{on}\, a\, d$


$V_o \sim k_{on}\ a\ d$
a = actin conc at membrane
d
d = size increment of one monomer


Leading
barbed end

The actin dynamics in the cell are not just simple treadmilling. The speed of motion of the cell is not consistent with treadmilling.

Coupling biochemistry and mechanics of motion

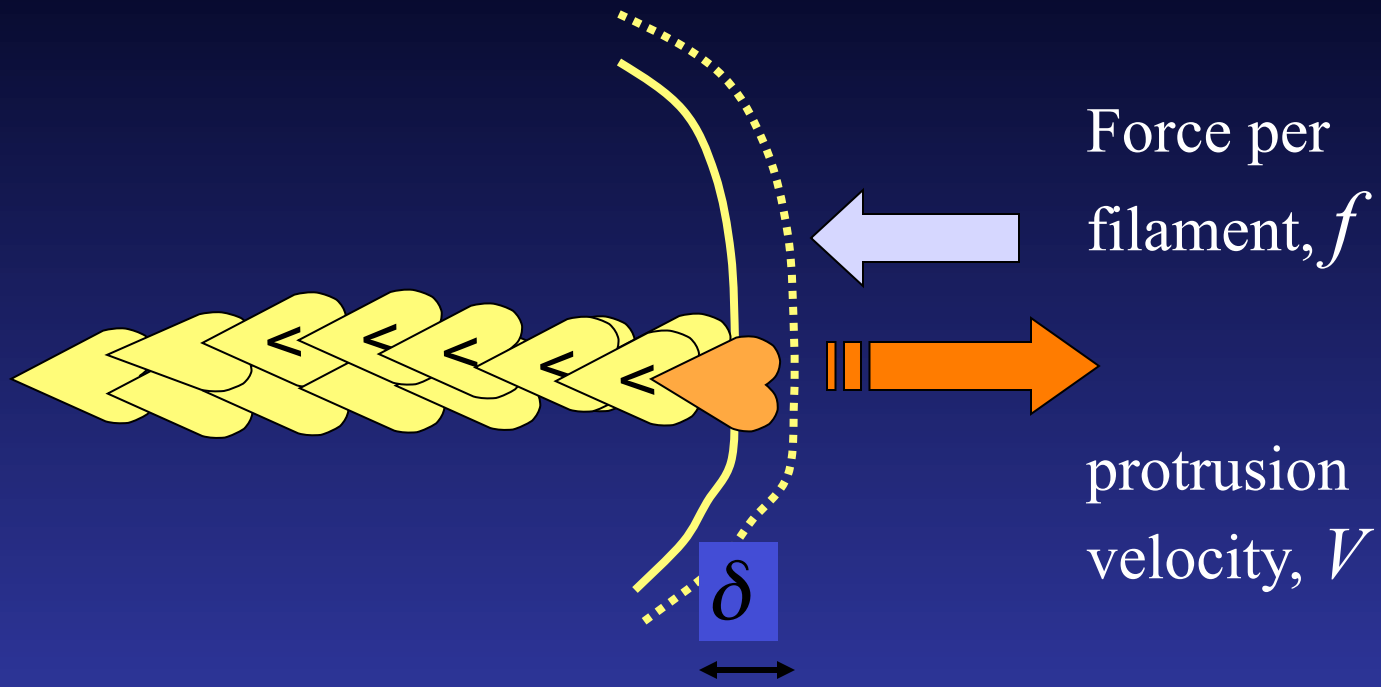
Mogilner & Oster (1996) Biophys J, 71: 3030-3045

The leading edge of the cell moves against a load force. How does the protrusion velocity depend on that force?

The Thermal Ratchet Model

Mogilner & Oster

Thermal fluctuations occasionally create a gap between the cell membrane and the tips of actin filaments. Monomers can fill in this gap to cause the displacement to persist.



Thermal Ratchet Model

Mogilner & Oster

Work done to create gap

Thermal energy

$$\frac{\delta f}{k_B T}$$

Speed of motion of one filament barbed end

$$V \approx \delta \ (k_{on} a \, e^{-\delta f / k_B T} - k_{off})$$

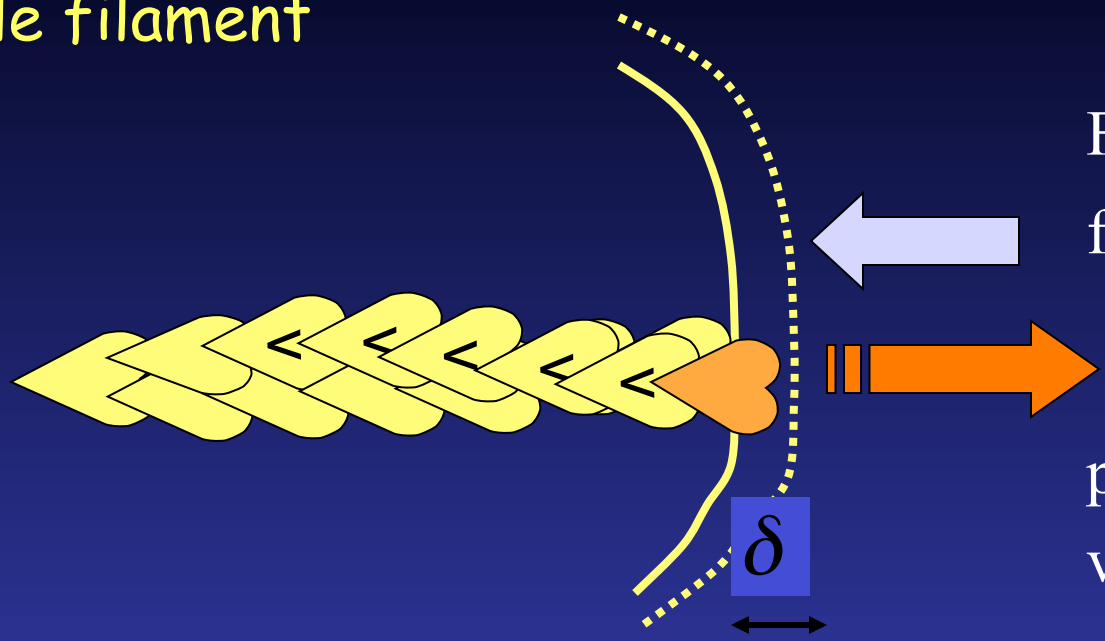
on rate

Probability of a gap forming

off rate

(very small)

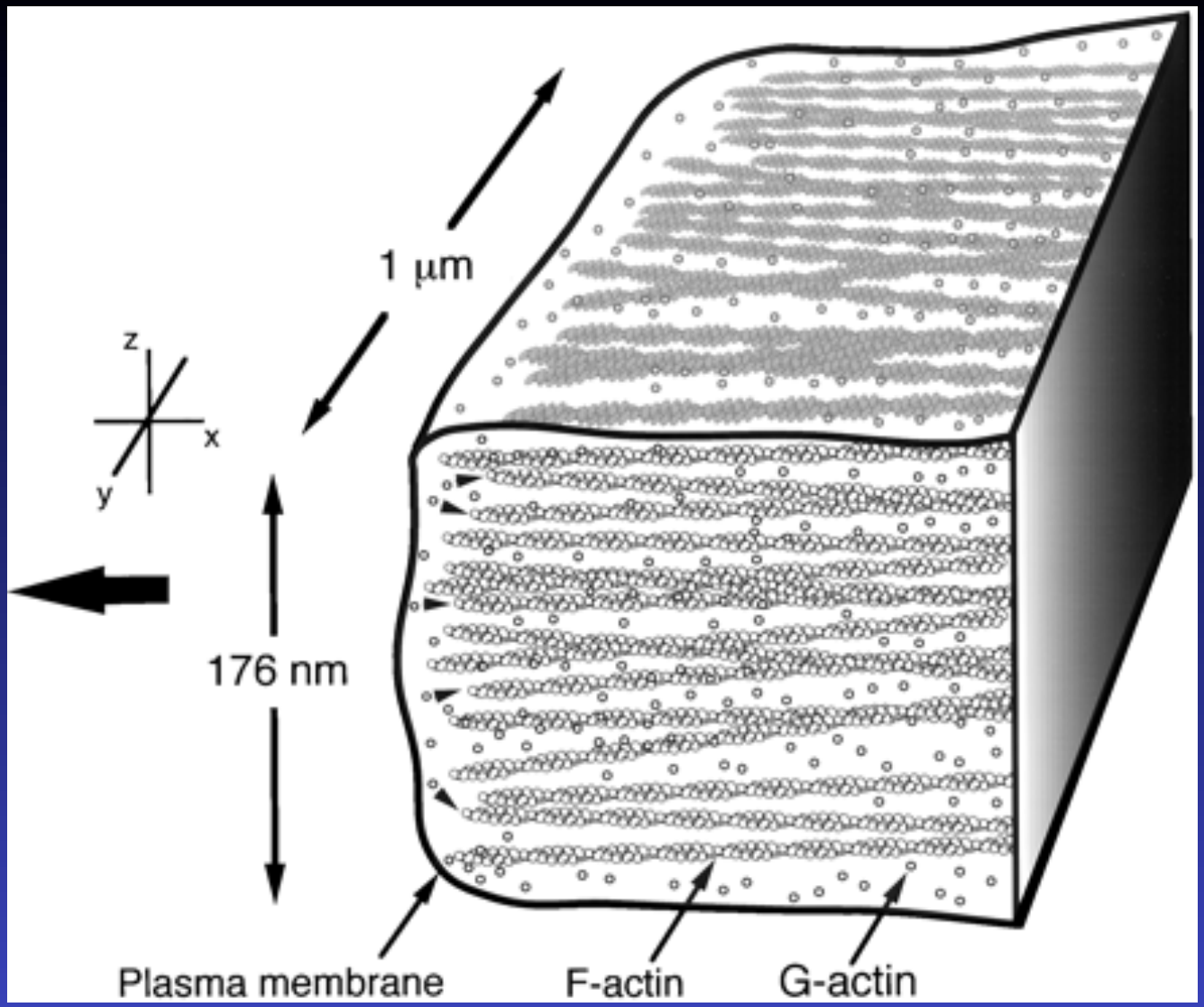
Load-Velocity relation for single filament



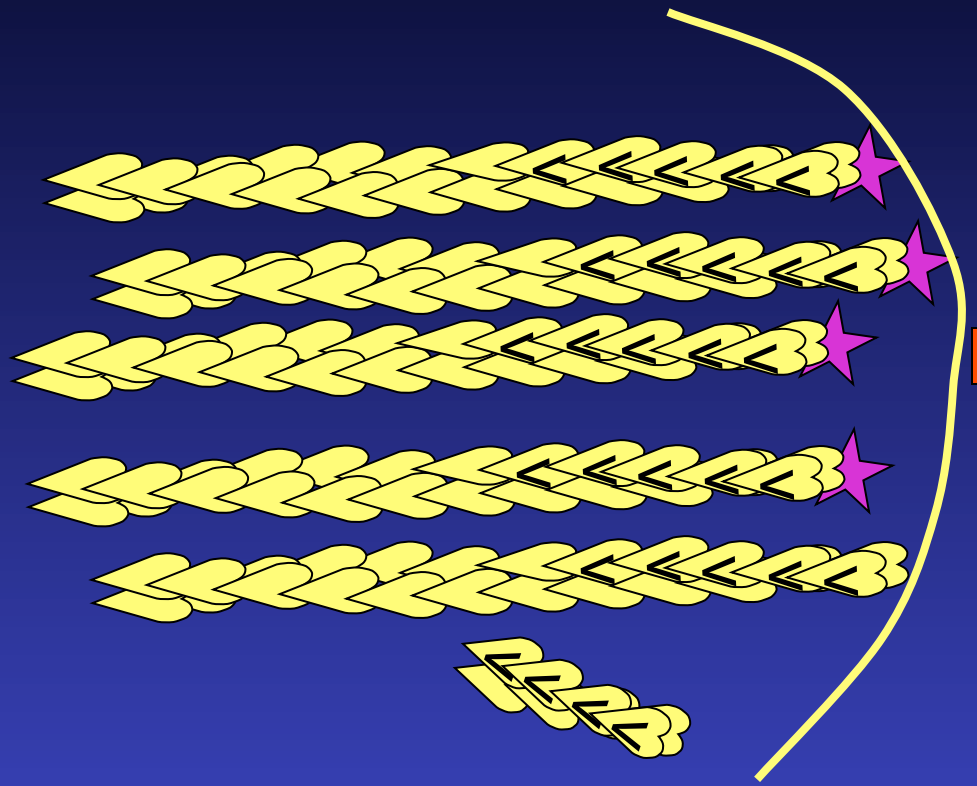
$$V \approx V_0 \exp(-\delta f / k_B T)$$

- Free polymerization velocity
- Monomer size
- Load force
- Thermal energy

Actin filaments and cell motility



Abraham, Krishnamurthi, Taylor, Lanni (1999) Biophys J 77: 1721-1732



There are many filament barbed ends at the cell membrane.

Only some can participate in motion.

The others are capped.

How does the protrusion velocity depend on the number of barbed ends ?

Length of actin monomer	2.72 nm	Abraham et al 1999
thermal energy kT	4.1 pN nm	Peskin et al 1993
actin monomer on-rate	11.6 /μM /s	Pollard 1986
actin monomer off-rate	1.4/s	Pollard 1986
b-end capping rate	4 /s	Schafer et al 1996
Arp2/3 attachment rate	1-10 /s	speculative
Arp2/3 diffusion coef	3 μ^2/s	calculated
length of lamellipod	~ 10 μ	Abraham et al 1999
thickness of lamellipod	0.1 μ	Abraham et al 1999
number b-ends at margin	240/μ	Abraham et al 1999
monomers in 1 μM actin	600/μ^3	conversion factor

Growth factors activate cell-surface receptors which signal the family of WASp/Scar proteins.

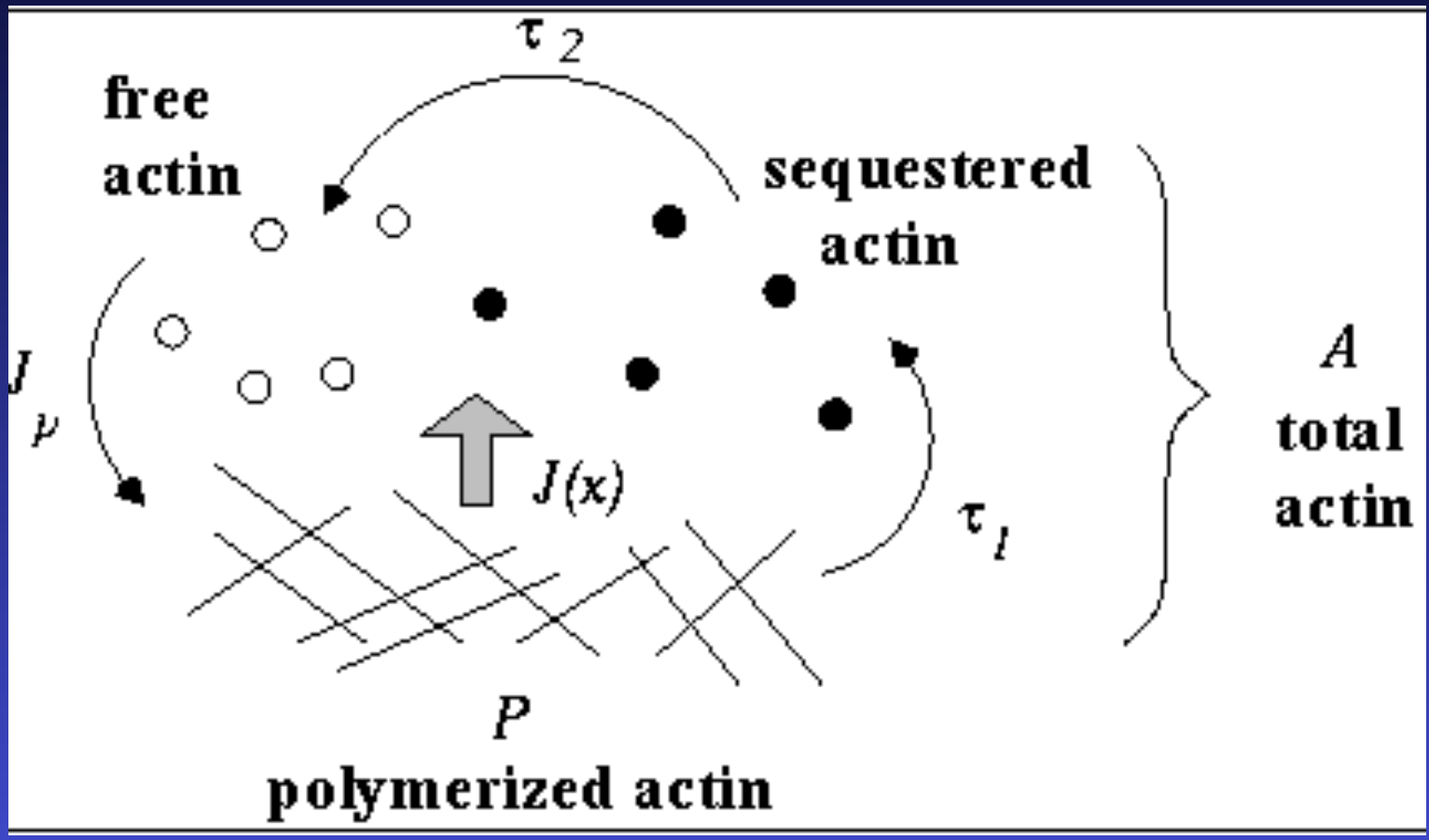
These activate Arp2/3 complexes

The Arp2/3 complex attaches to some pre-existing actin filament and initiates a new actin filament

How many uncapped barbed ends should optimally be kept active (uncapped) and available for growth at the leading edge ?

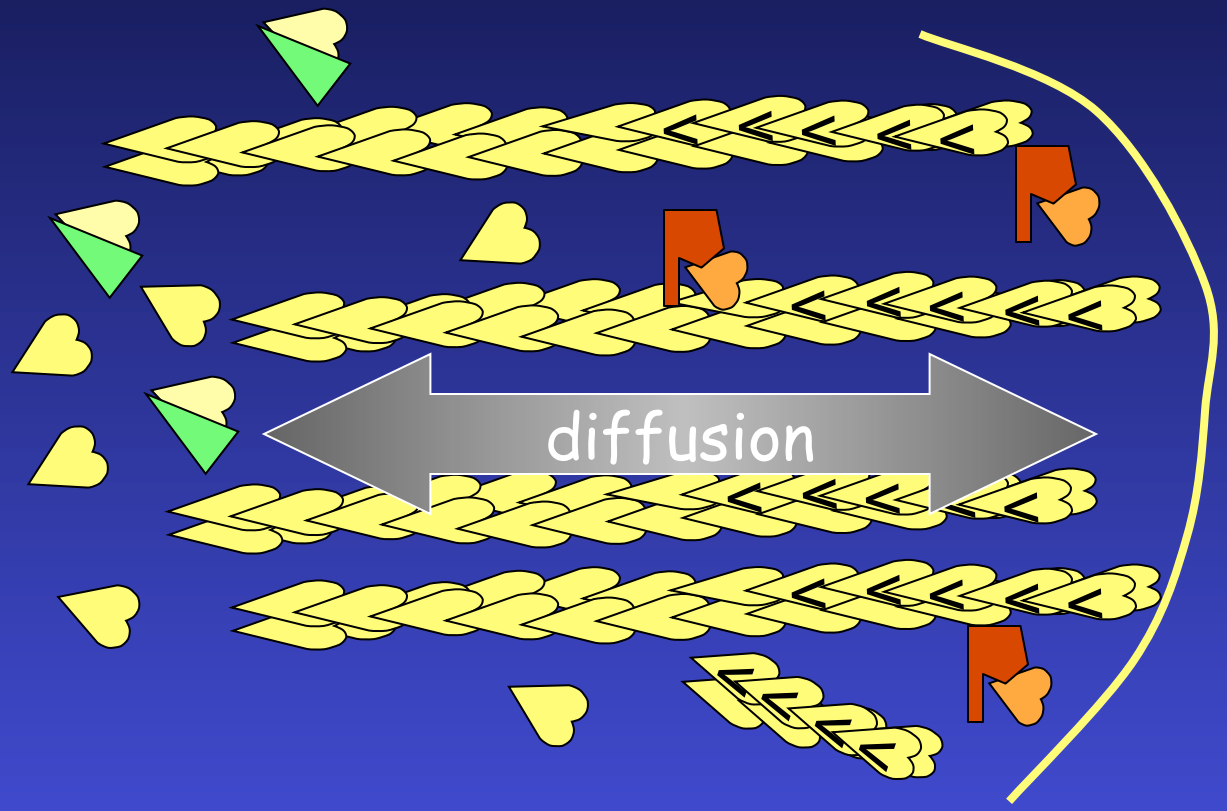
Ingredients of the model:

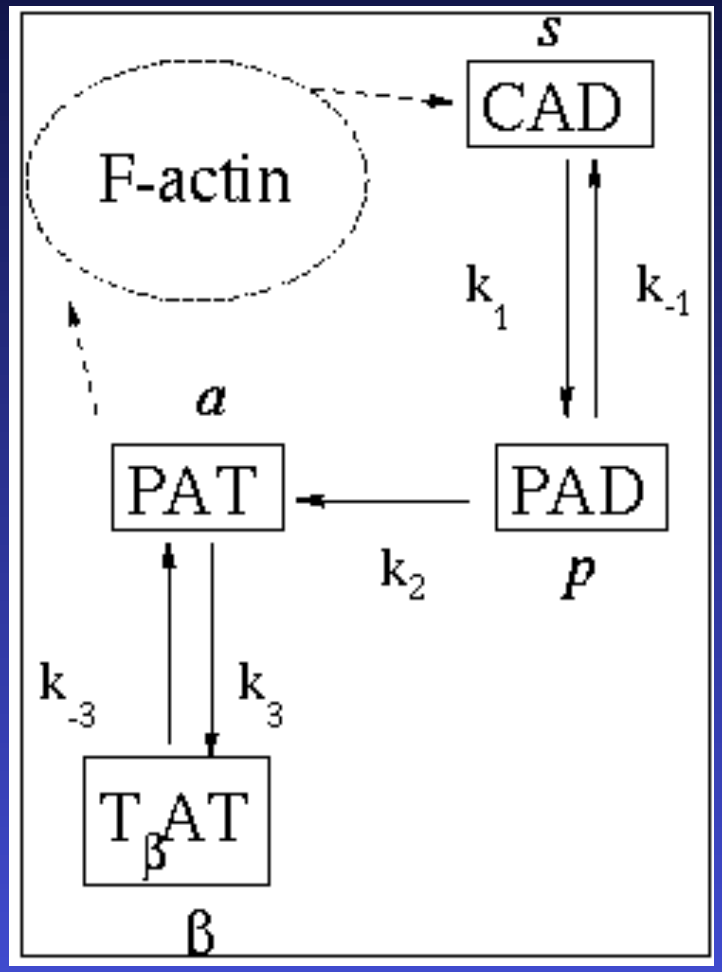
(1) Actin monomer cycle



Ingredients of the model:

(2) Diffusion of actin monomers in various forms across the lamellipod





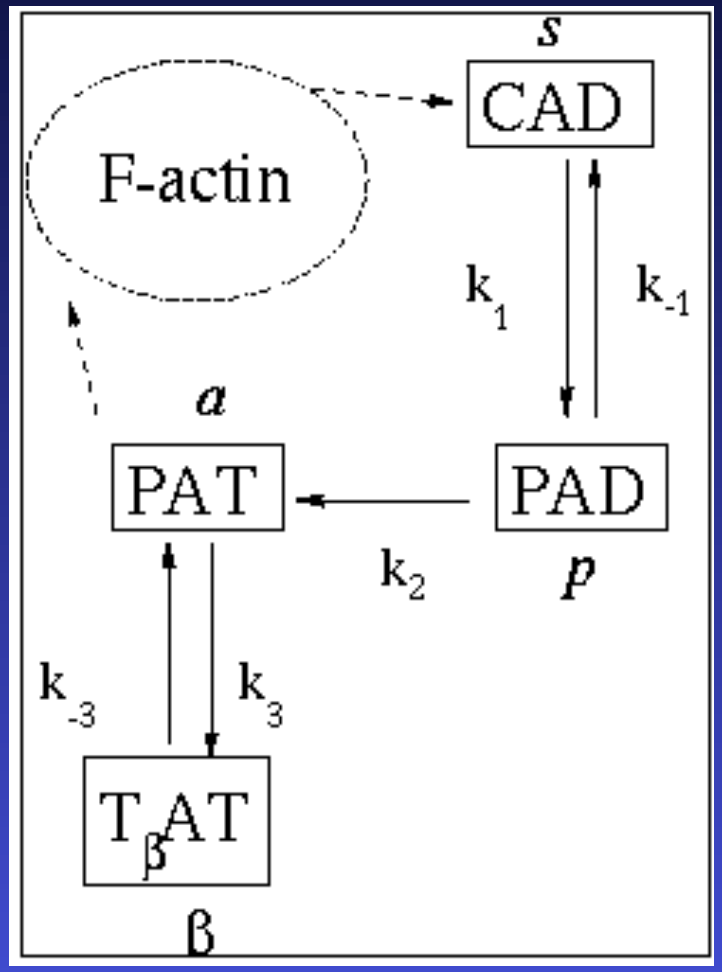
S=ADP-G-actin-cofilin CAD

p=ADP-G-actin-profilin PAD

a=ATP-G-actin-profilin PAT

B=ATP-G-actin-thymosin TAT

Balance equations



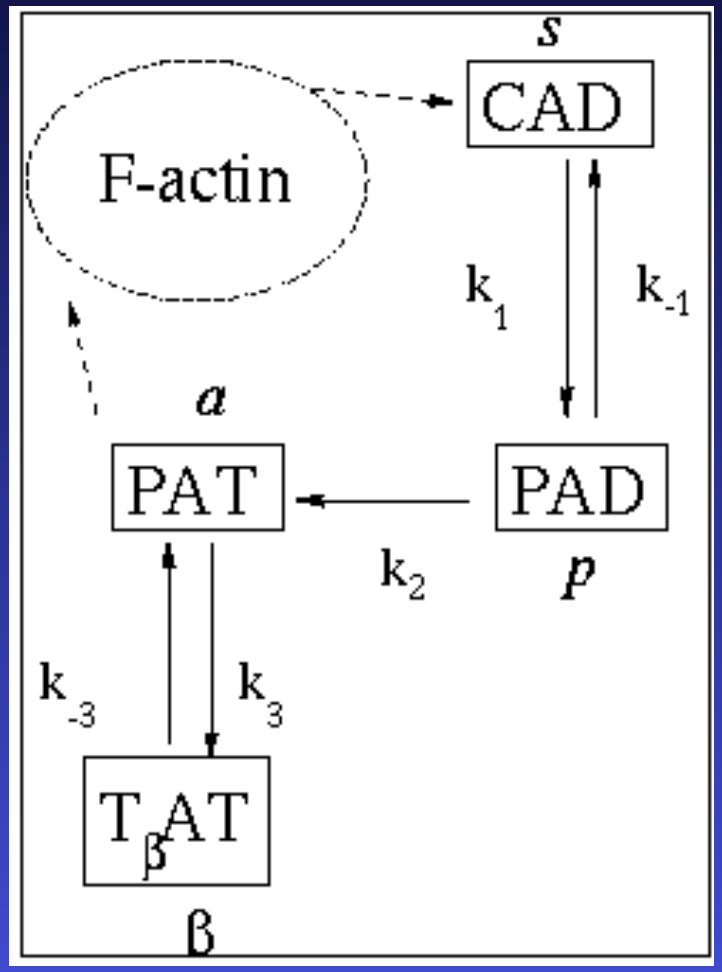
$$\frac{\partial s}{\partial t} = -V\frac{\partial s}{\partial x} + D\frac{\partial^2 s}{\partial x^2} - k_1 s + k_{-1} p + J_d(x),$$

$$\frac{\partial p}{\partial t} = -V\frac{\partial p}{\partial x} + D\frac{\partial^2 p}{\partial x^2} + k_1 s - k_{-1} p - k_2 p,$$

$$\frac{\partial \beta}{\partial t} = -V\frac{\partial \beta}{\partial x} + D\frac{\partial^2 \beta}{\partial x^2} - k_{-3}\beta + k_3 a,$$

$$\frac{\partial a}{\partial t} = -V\frac{\partial a}{\partial x} + D\frac{\partial^2 a}{\partial x^2} + k_{-3}\beta - k_3 a + k_2 p.$$

Steady state



$$
\begin{aligned}
D\frac{d^2 s}{dx^2} - k_1 s + k_{-1} p + J_d(x) &= 0, \\
D\frac{d^2 p}{dx^2} + k_1 s - k_{-1} p + k_2 p &= 0, \\
D\frac{d^2 \beta}{dx^2} - k_{-3}\beta + k_3 a &= 0, \\
D\frac{d^2 a}{dx^2} + k_{-3}\beta - k_3 a + k_2 p &= 0.
\end{aligned}
$$

Full set of ODEs and BCs

$$D\frac{d^2s}{dx^2} - k_1 s + k_{-1} p + J_d(x) = 0$$

$$\left.\frac{ds}{dx}\right|_{x=0} = \left.\frac{ds}{dx}\right|_{x=L} = 0$$

$$D\frac{d^2p}{dx^2} + k_1 s - k_{-1} p - k_2 p = 0$$

$$\left.\frac{dp}{dx}\right|_{x=0} = \left.\frac{dp}{dx}\right|_{x=L} = 0$$

$$D\frac{d^2\beta}{dx^2} - k_{-3}\beta + k_3 a = 0$$

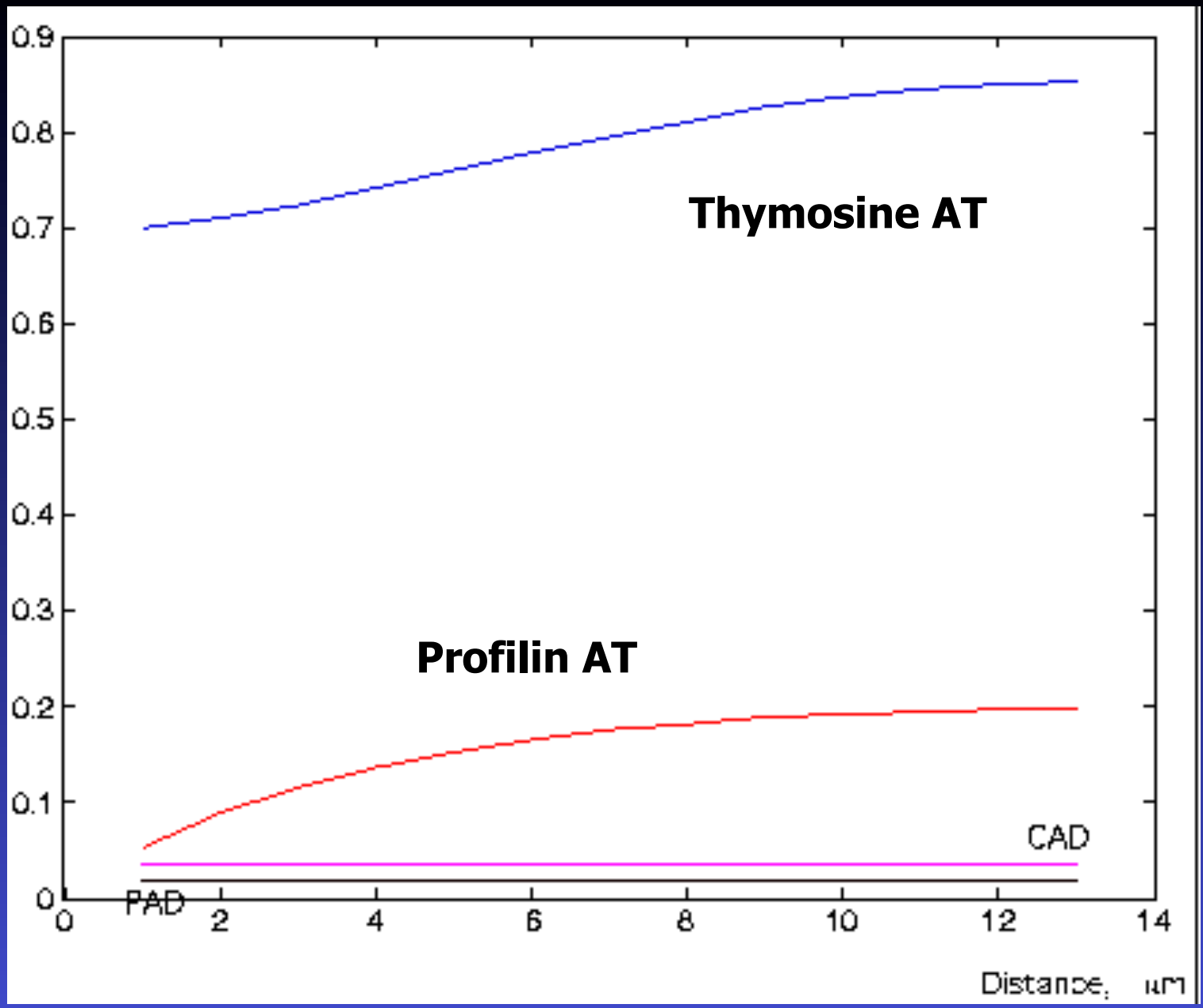
$$\left.\frac{d\beta}{dx}\right|_{x=0} = \left.\frac{d\beta}{dx}\right|_{x=L} = 0$$

Actin monomer boundary condition

$$\left(-D\frac{\partial a}{\partial x}+Va\right)\Big|_{x=0}=-J_p=-VB/\delta\eta.$$

Flux of actin monomers to the cell edge

Growth of the barbed ends at the cell edge


Thymosine AT
Profilin AT
CAD
PAD
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
14
Distance, um

From the model and biological parameter values, we determine the actin monomer concentration available at membrane to drive protrusion.

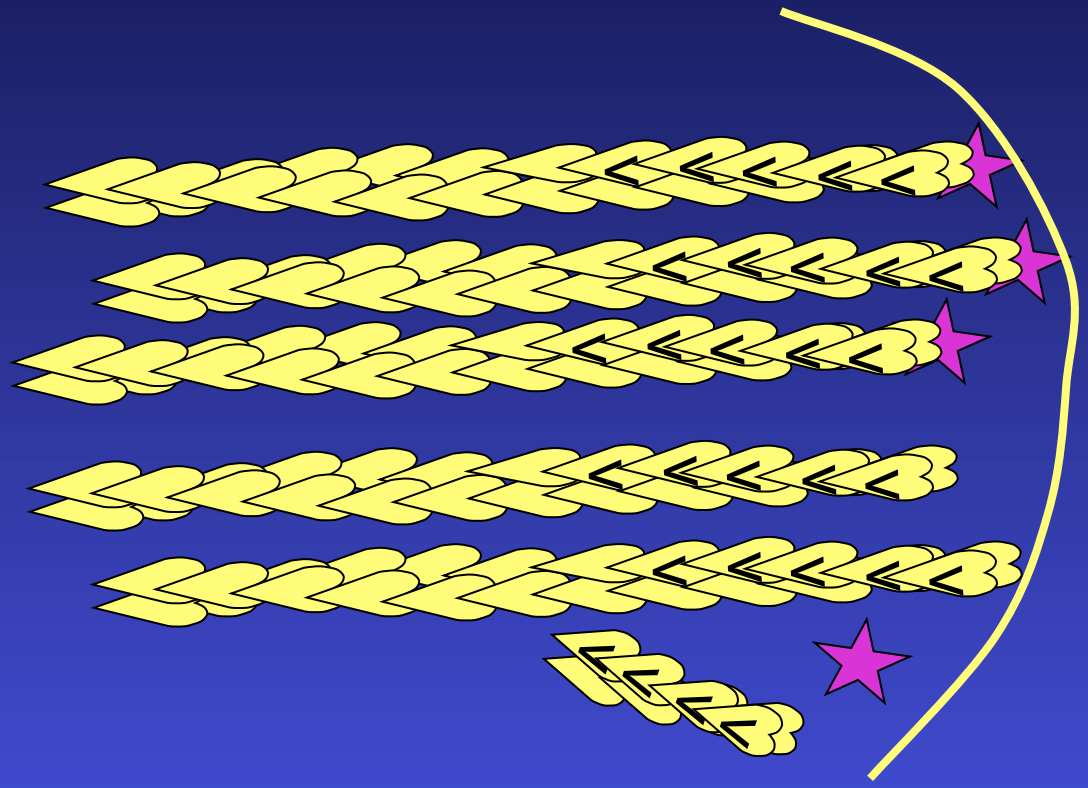
$$a(0) = \frac{k_{-3}}{k_3 + k_{-3}} \left(A - \frac{J_p \tau}{L} \right),$$

$$\tau = \tau_{dep} + \tau_{cof} + \tau_{rec}, \quad \tau_{dep} = 1/r,$$

$$\tau_{cof} = \frac{k_1 + k_{-1} + k_2}{k_1 k_2},$$

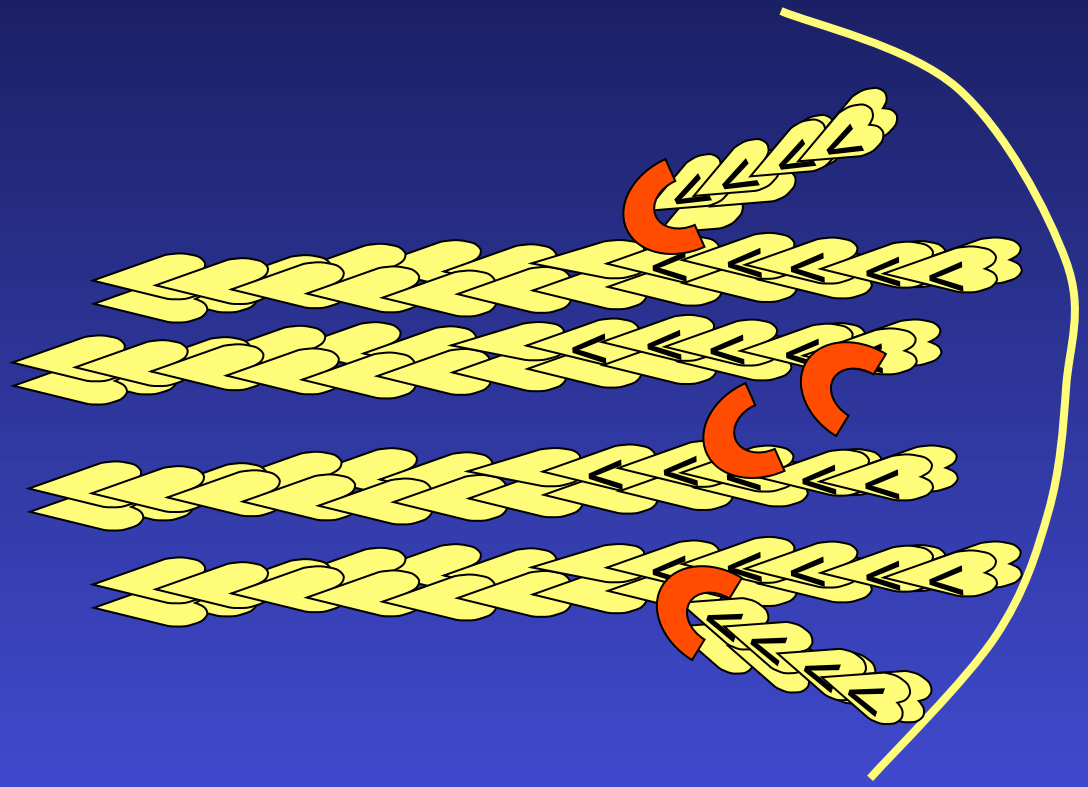
Ingredients of the model:

(3) Capping/uncapping of the filament ends



Ingredients of the model:

(4) Nucleation of new ends by Arp2/3



Ingredients of the model:

(5) Motion of barbed ends at and close to membrane

$$\frac{dB}{dt} = n - \gamma B.$$

nucleation

capping

Protrusion Velocity

$$V_0 = k_{on}\delta a(0).$$

If no lead force, this is the rate of protrusion

$$V = \delta(k_{on}a(0)e^{-\delta f/k_B T} - k_{off}),$$

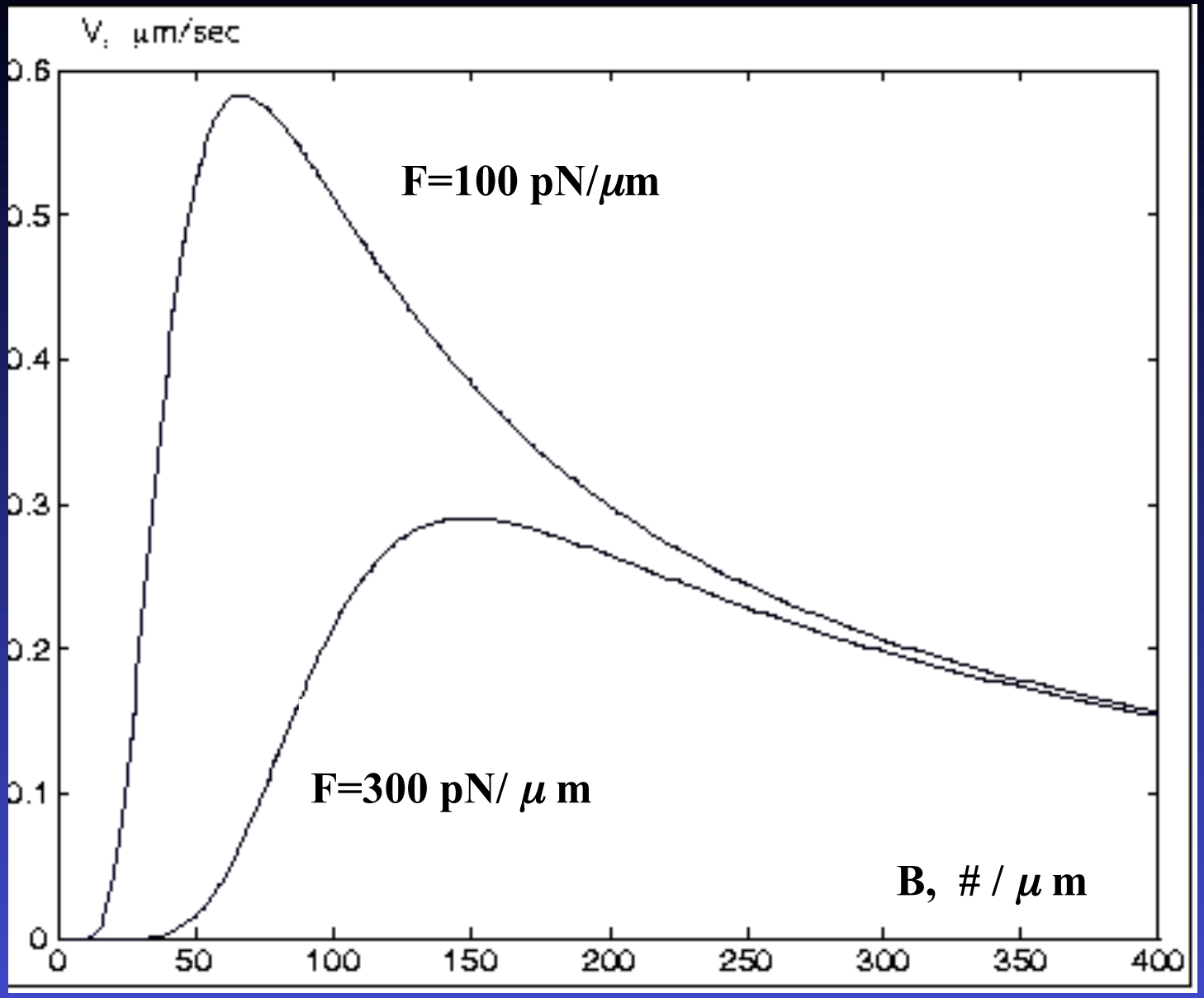
$$V \simeq V_0 e^{-\delta f/k_B T}.$$

Result:

Protrusion velocity depends on kinetic rate constants and on the number of barbed ends (B) pushing the membrane

$$V = \frac{\bar{V}}{\kappa \exp(w/B) + \alpha B},$$

$$\bar{V} = k_{on}\delta A, \quad \kappa = \left(1 + \frac{k_3}{k_{-3}}\right), \quad \alpha = \left(\frac{k_{on}\tau}{\eta L}\right) \quad w = F\delta/k_B T.$$


V, μm/sec
0.6
0.5
0.4
0.3
0.2
0.1
0
0
50
100
150
200
250
300
350
400
F=100 pN/μm
F=300 pN/ μ m
B, # / μ m

The model predicts:

- There is an optimal barbed end density
- (This also means that there are optimal nucleation and capping rates)
- the protrusion drops very rapidly for barbed ends below their optimal density, but drops more gradually above the optimum

This suggests that careful regulation of the barbed ends is needed in the cell.

Further model predictions:

- A greater amount of total actin and a faster rate of actin turnover correlate positively with the rate of locomotion.
- Increasing the amount of thymosin slows down locomotion

How many uncapped barbed ends should be kept available for growth at the leading edge ?

- too few: force to drive protrusion insufficient.

- too many: competition for monomers depletes monomer pool too quickly, slowing growth.

Recent revision