Mathematical Cell Biology Graduate Summer Course

University of British Columbia, May 1-31, 2012

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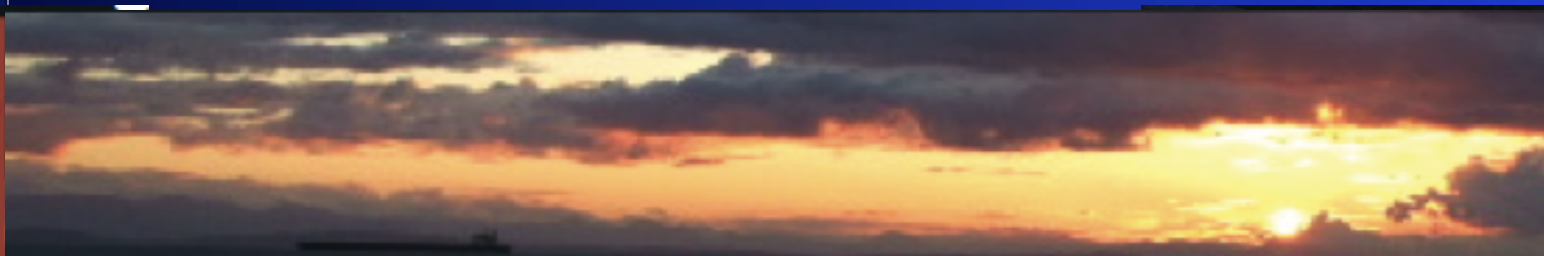
Introduction to Polymerization Kinetics



mprime

PIMS

www.math.ubc.ca/~keshet/MCB2012/

Perspectives

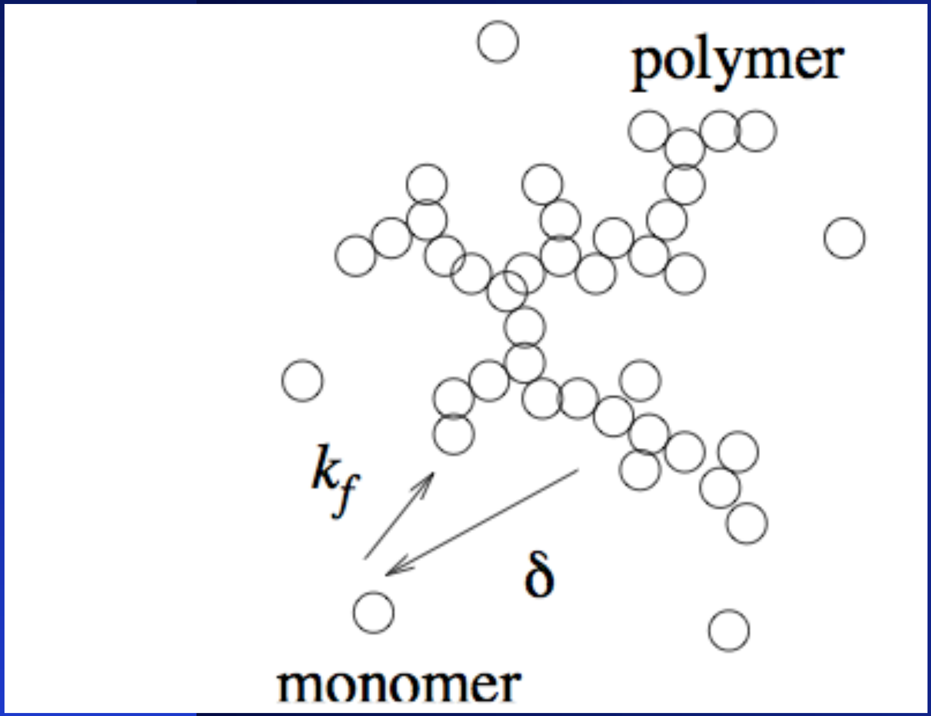
The cytoskeletal biopolymers are largely semi-rigid rods on typical size scale of cells.

We here examine their assembly kinetics in free polymerization mixtures.

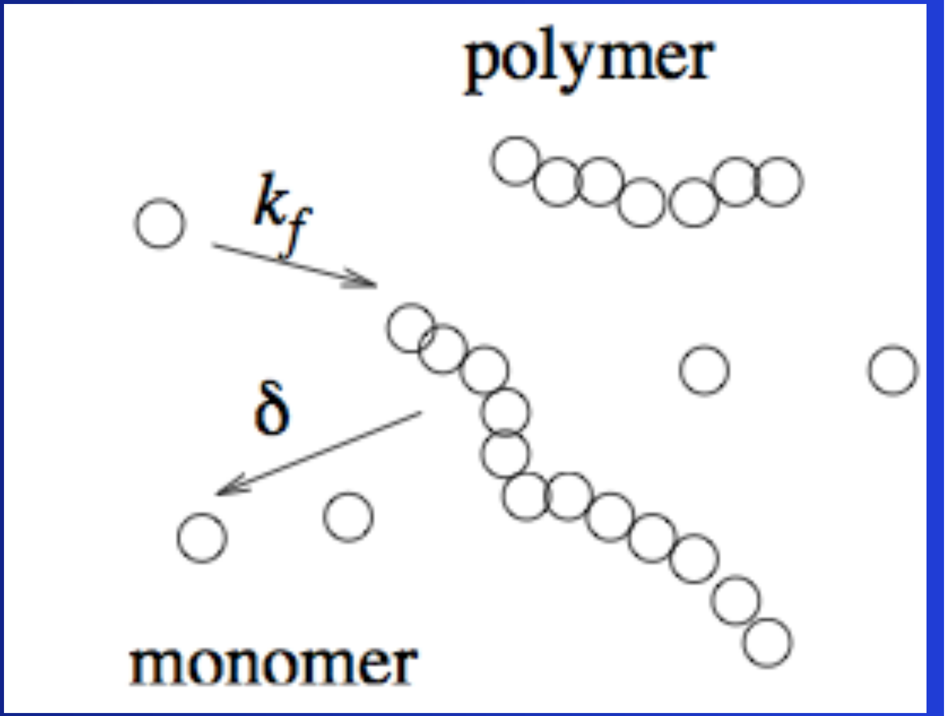
Addition or loss of a single monomer at each step

Contrasting two types of polymerization events

Simple aggregation



Extension at polymer ends



Variables

$$c(t) = \text{number of monomer subunits in the volume at time } t,$$
$$F(t) = \text{amount of polymer (in number of monomer equivalents) at time } t,$$
$$A(t) = \text{total amount of material (in number of monomer equivalents) at time } t.$$

$n =$ Number of filaments (or filament tips) at which polymerization can occur.

Typical models

Simple aggregation

$$\frac{dc}{dt} = -k_f cF + \delta F,$$
$$\frac{dF}{dt} = k_f cF - \delta F.$$

Extension at polymer ends

$$\frac{dc}{dt} = -k_f cn + \delta F,$$
$$\frac{dF}{dt} = k_f cn - \delta F.$$

Conservation

Total amount $A = c + F$ is constant

Example:

Simple aggregation

$$\frac{dc}{dt} = -k_f cF + \delta F,$$
$$\frac{dF}{dt} = k_f cF - \delta F.$$

Eliminate F and explain what behaviour you expect to see for *c(t)*

Total amount $A = c + F$ is constant

Miniproblem

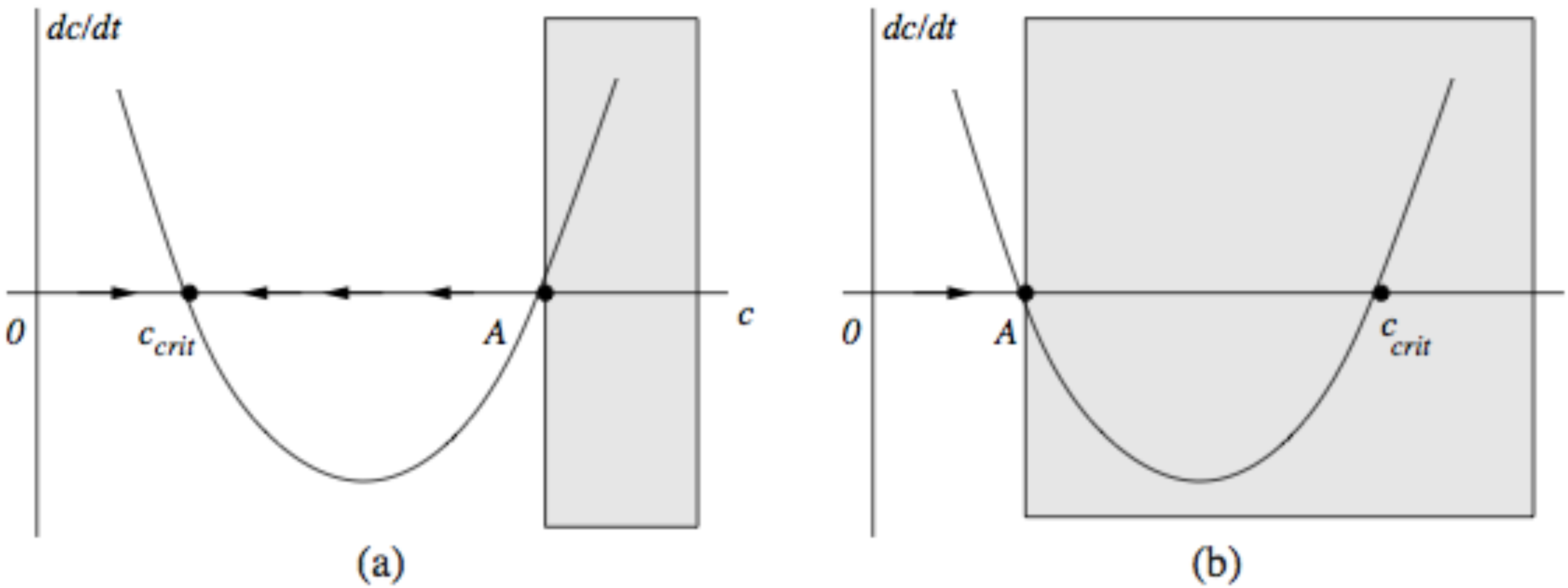
$$\frac{dc}{dt} = -k_f cF + \delta F,$$
$$\frac{dF}{dt} = k_f cF - \delta F.$$

- Find the critical concentration needed in order for polymerization to take place.

Predictions

$$\frac{dc}{dt} = k_f(A-c)(c_{crit}-c).$$

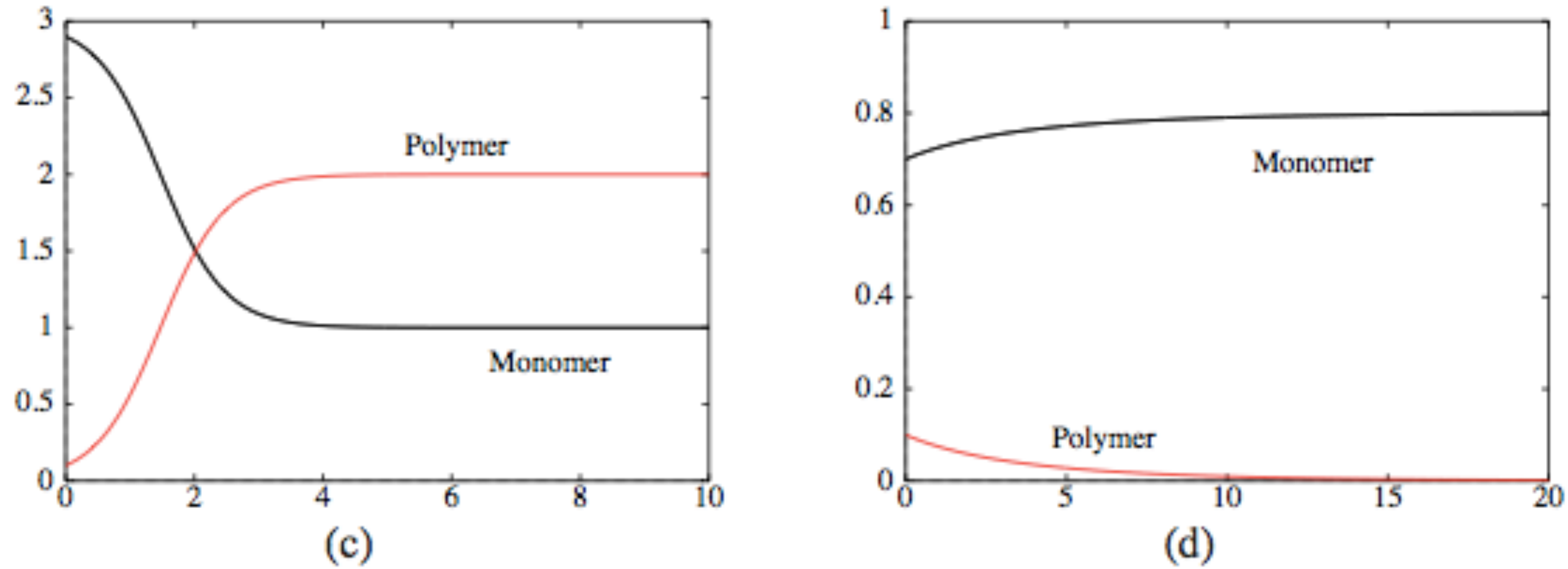
$$c_{crit} = \delta/k_f$$



$A > c_{crit}$

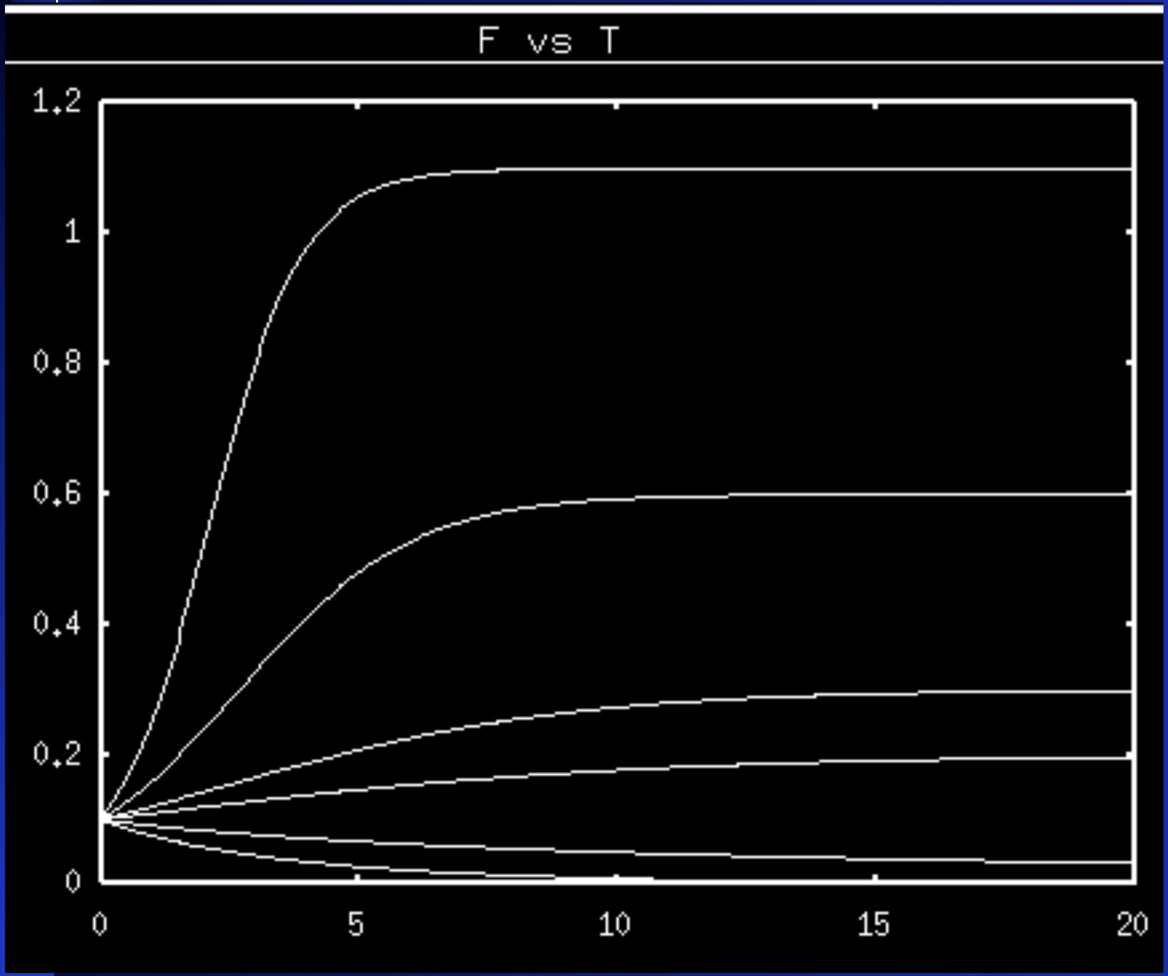
$A < c_{crit}$

Critical monomer concentration



Polymer will only form if total amount (A) > critical level

“Experimental polymerization curves”

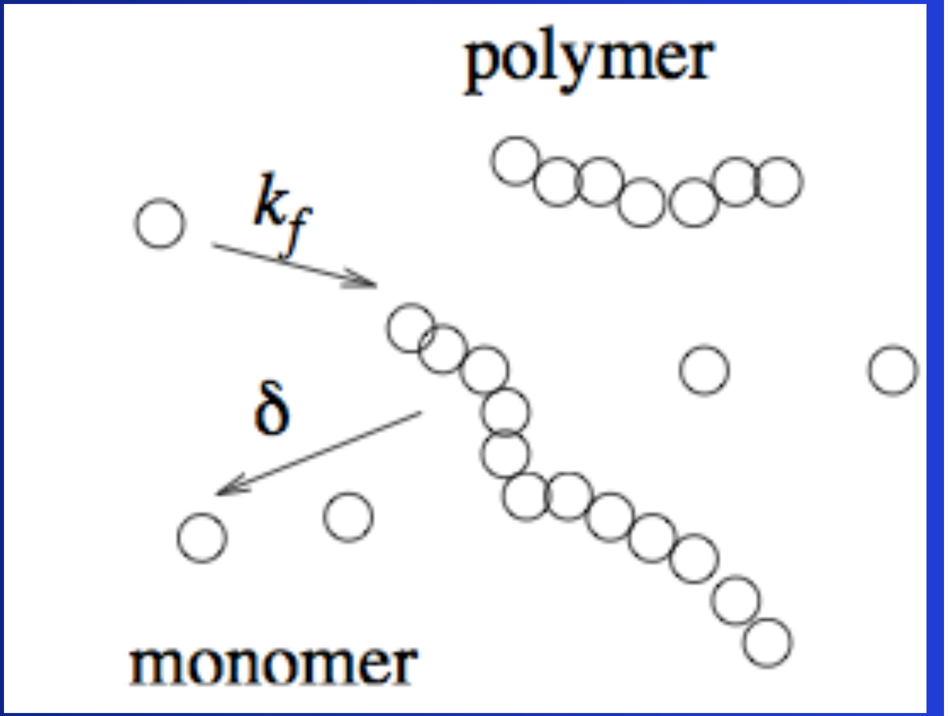


Varying c(0) will affect

(a) Whether polymer persists
(b) Time course
(c) Maximal level of polymer formed

Second type of polymer: different behaviour

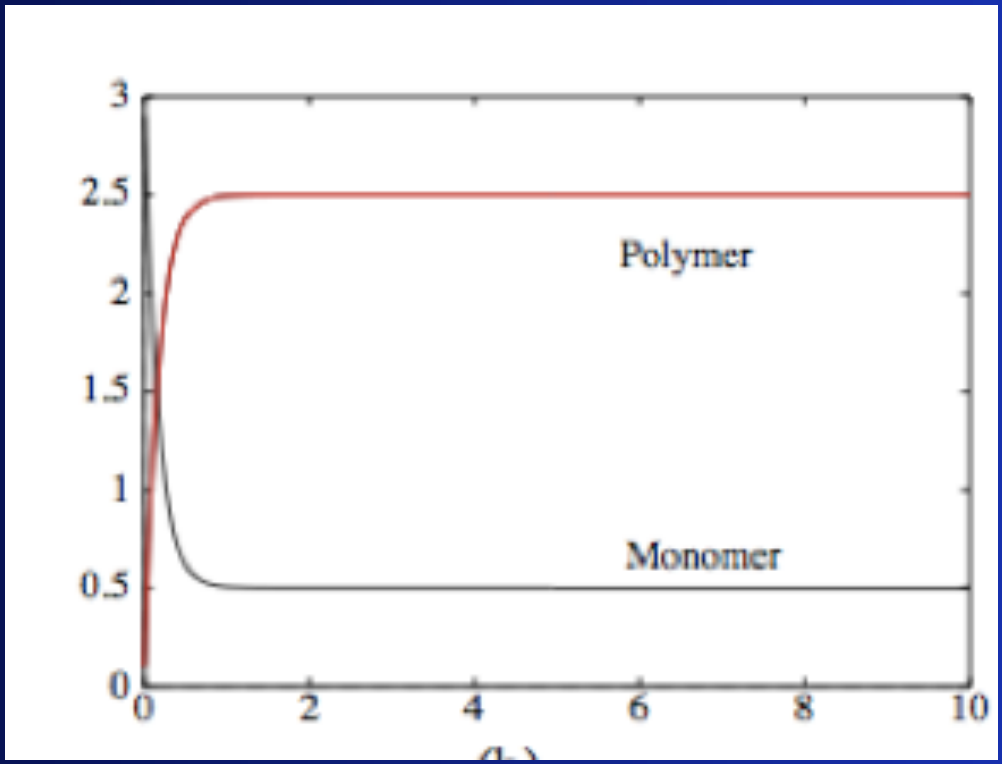
Extension at polymer ends



Here monomers can only be added at the ends of the filament.

Observe: the kinetics are different!

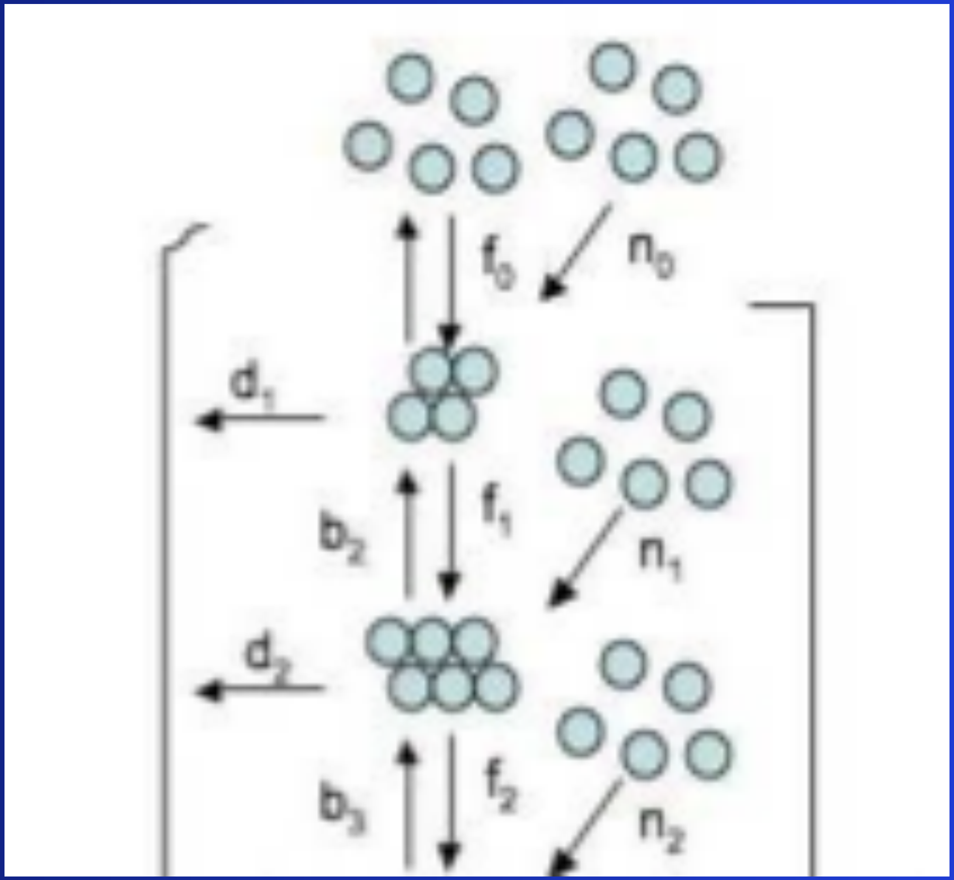
Equilibrium levels



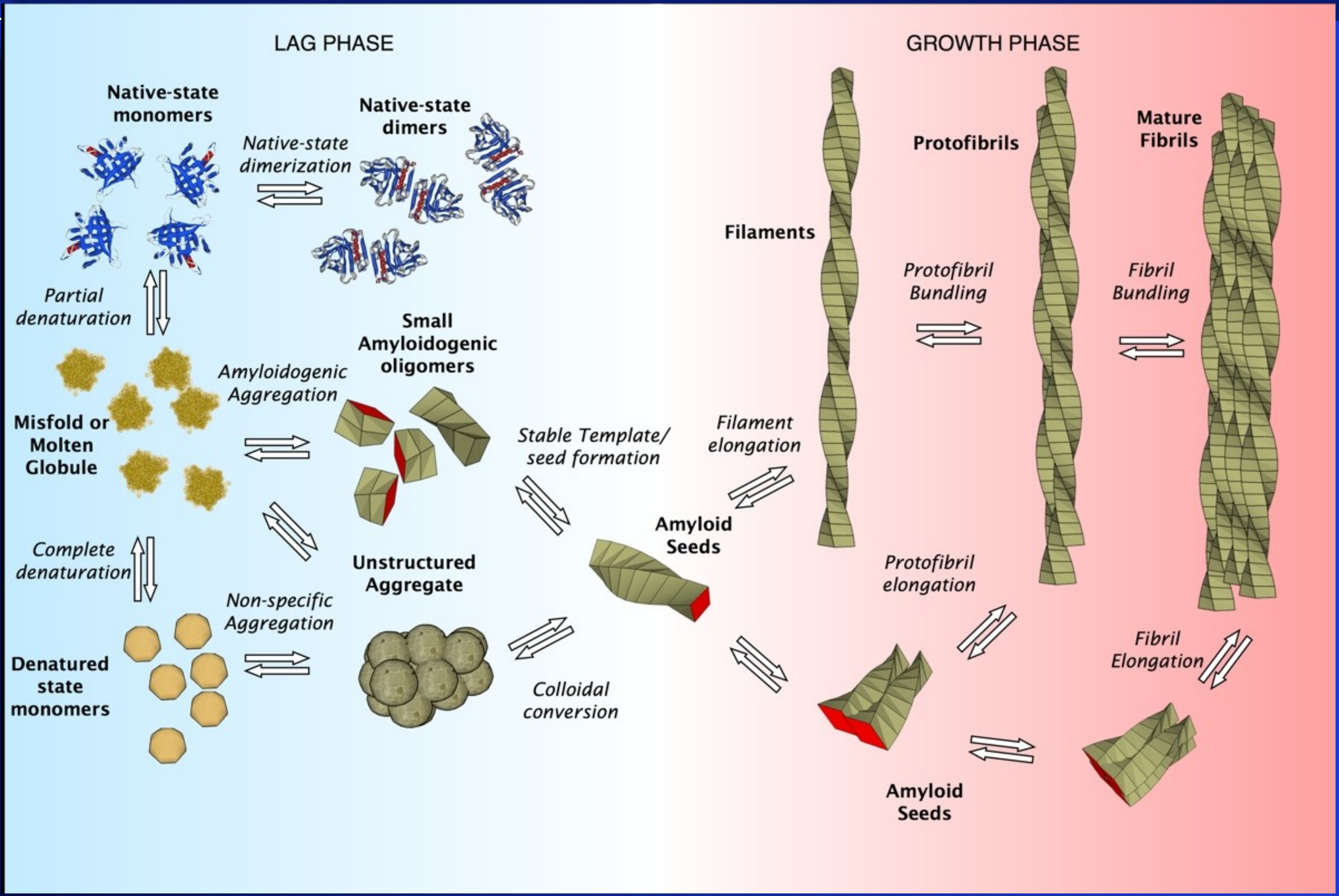
In this example there is always some equilibrium ratio of polymer and monomer.

Some polymers grow differently

- Addition of multiple subunits at each initiation step.. Up to some stable nucleus size.



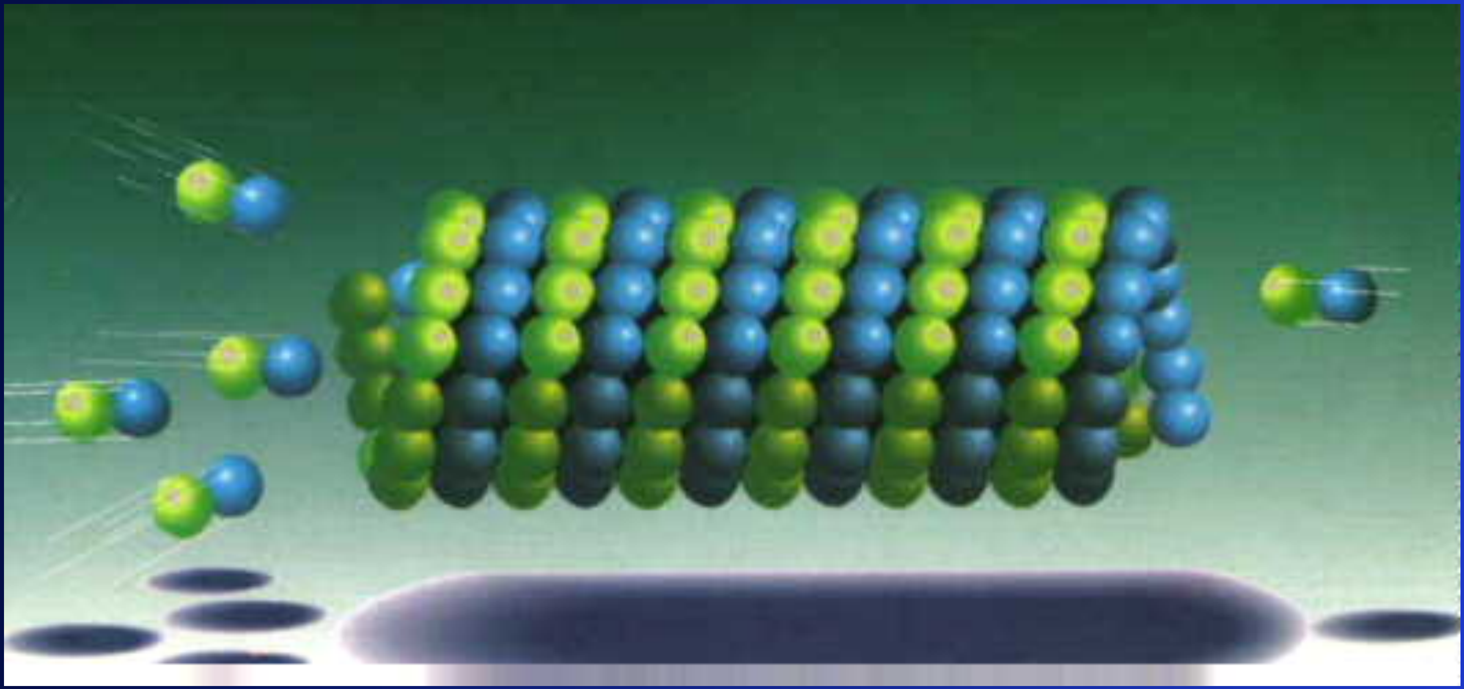
A huge variety of possibilities



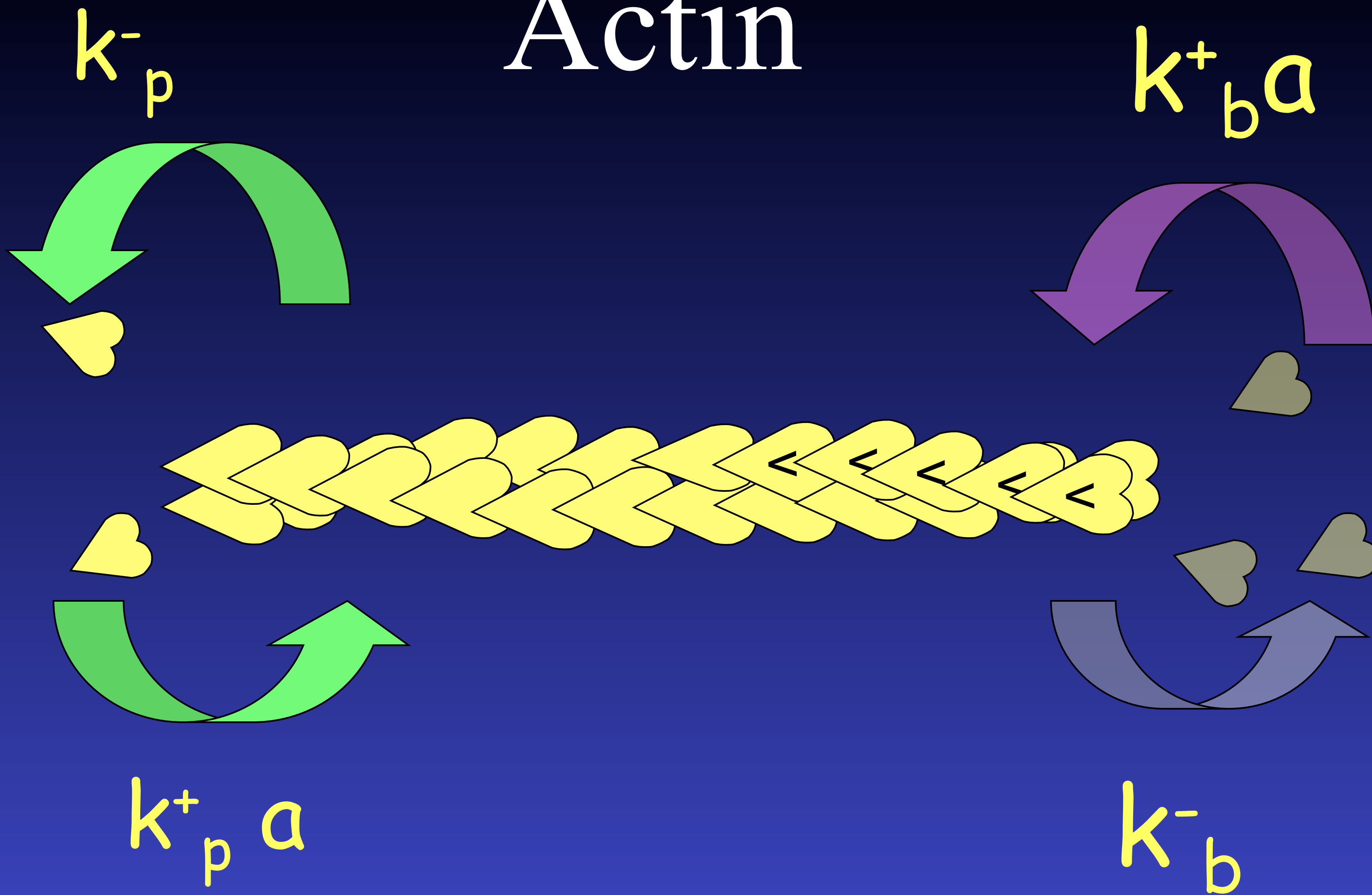
Cytoskeleton proteins

Actin filament:

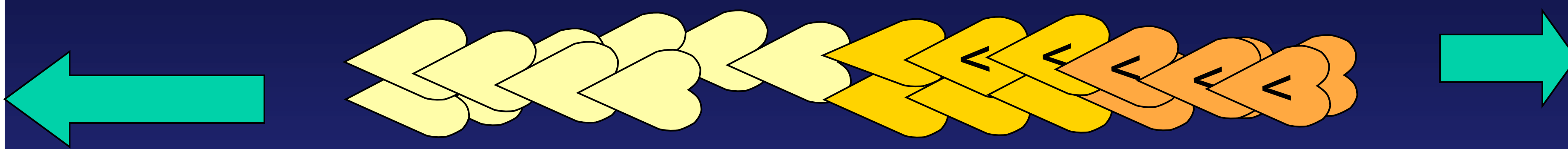
Microtubule:



Actin



Rate of growth at barbed/pointed ends:



depends on the actin monomer concentration:

$$(k_p^+ a - k_p^-)$$

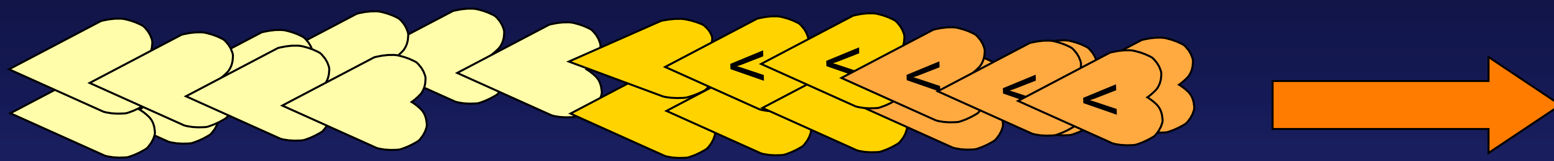
$$(k_b^+ a - k_b^-)$$

$$\frac{dl}{dt} = (k_b^+ a - k_b^-) + (k_p^+ a - k_p^-)$$

The treadmilling concentration:

Find the monomer concentration for which the length of the filament is constant because loss and gain of monomers exactly balance.

The treadmilling concentration:



$$a_{tread} = \frac{k_p^- + k_b^-}{k_p^+ + k_b^+} = \frac{k^-}{k^+}$$

At this concentration, monomers add to the barbed end at the same rate as they are lost at the pointed end.

Length of actin monomer	2.72 nm	Abraham et al 1999
actin monomer on-rate	11.6 /μM /s	Pollard 1986
actin monomer off-rate	1.4/s	Pollard 1986
number b-ends at margin	240/μ	Abraham et al 1999
monomers in 1 μM actin	600/μ^3	conversion factor

Why not so relevant for the cell:

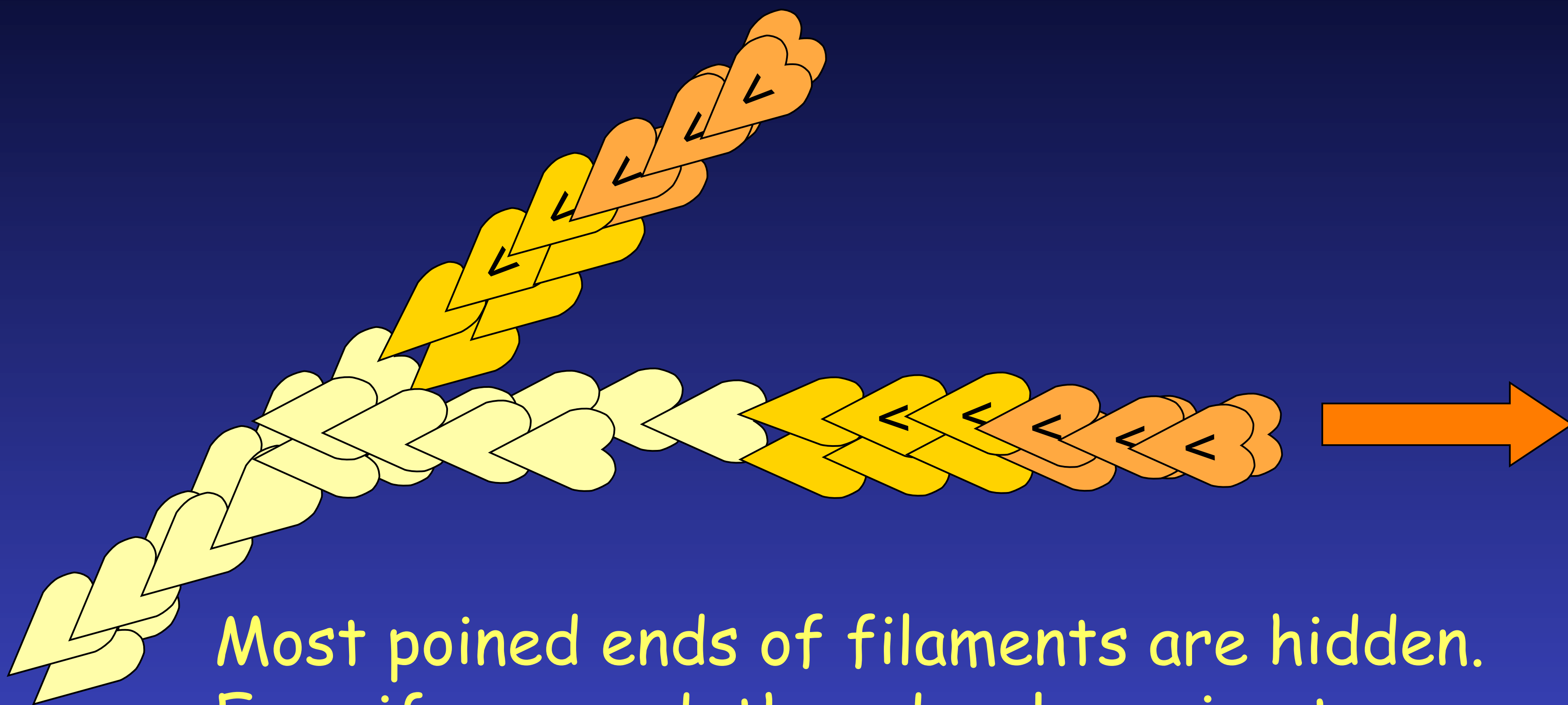
$k^{+} =$ 11.6 /μM /s Pollard 1986

$k^{-} =$ 1.4/s Pollard 1986

Then $a_{\text{tread}} \approx 0.12\ \mu\text{M}$

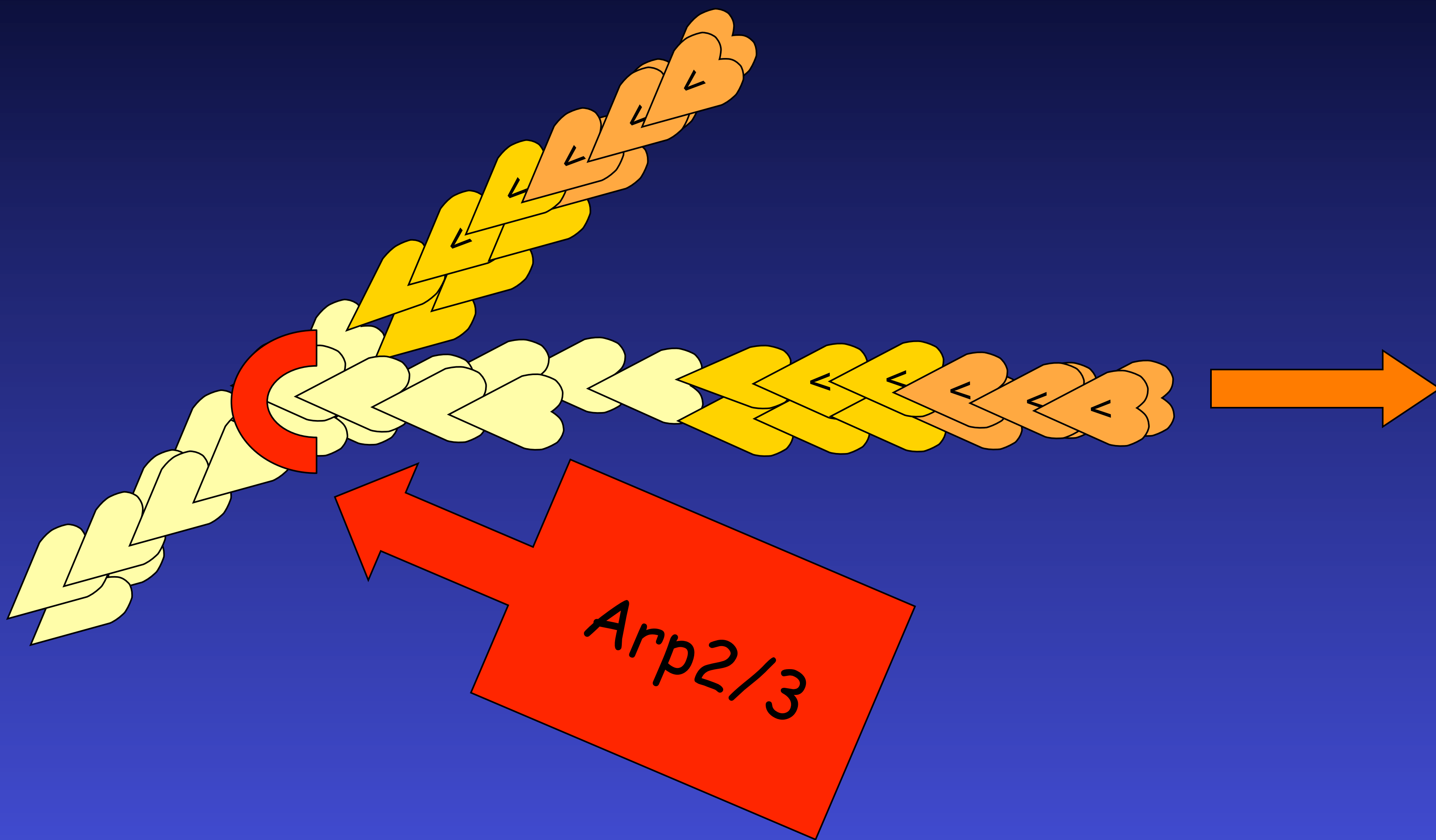
But in cell actin conc $\approx 20\ \mu\text{M}$

Treadmilling not so relevant in the cell

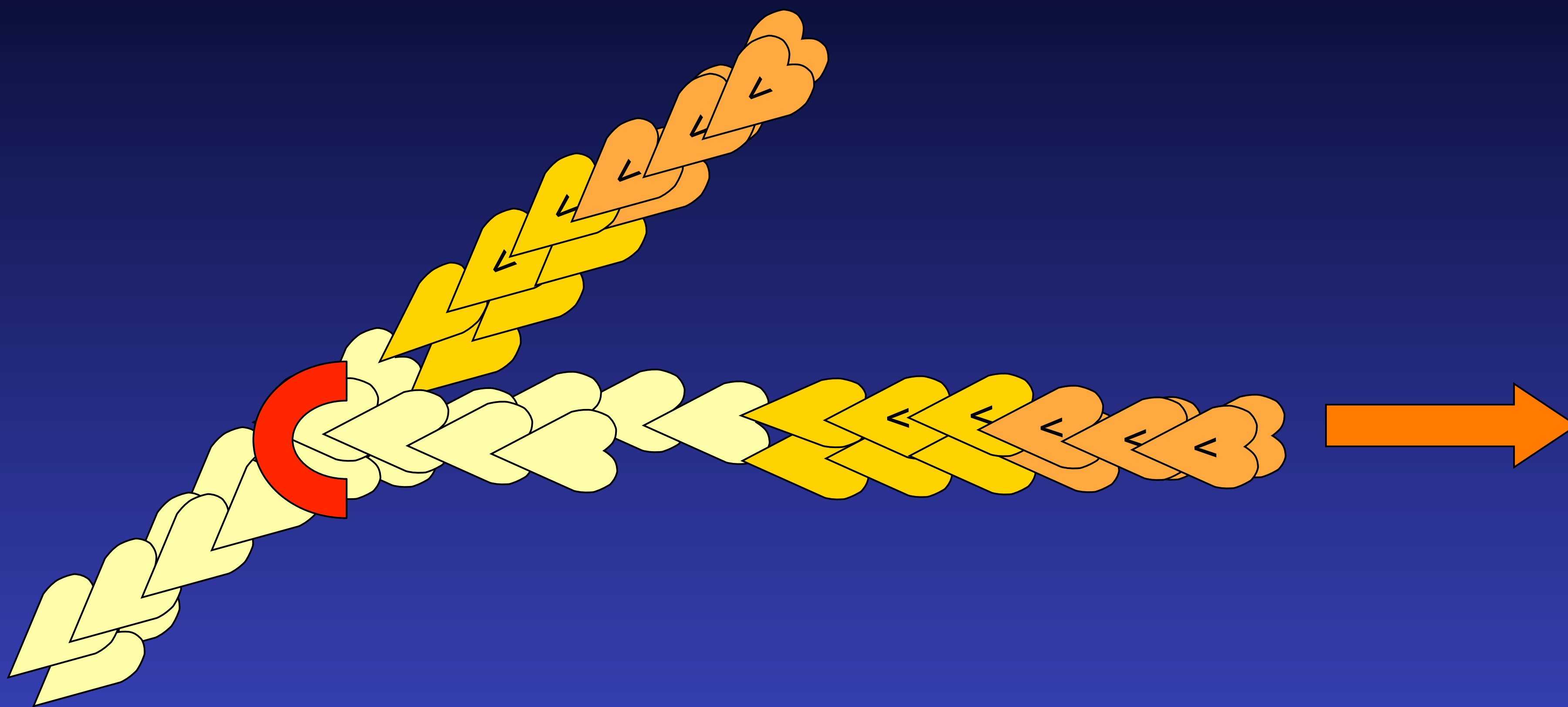


Most poined ends of filaments are hidden. Even if exposed, they depolymerize too slowly to keep up with growing barbed ends.

Actin filament branching:



Growth regulated by regulating nucleation of new barbed ends



This is done by regulating the amount of active Arp2/3

Signal -> WASP -> Arp2/3

stimuli

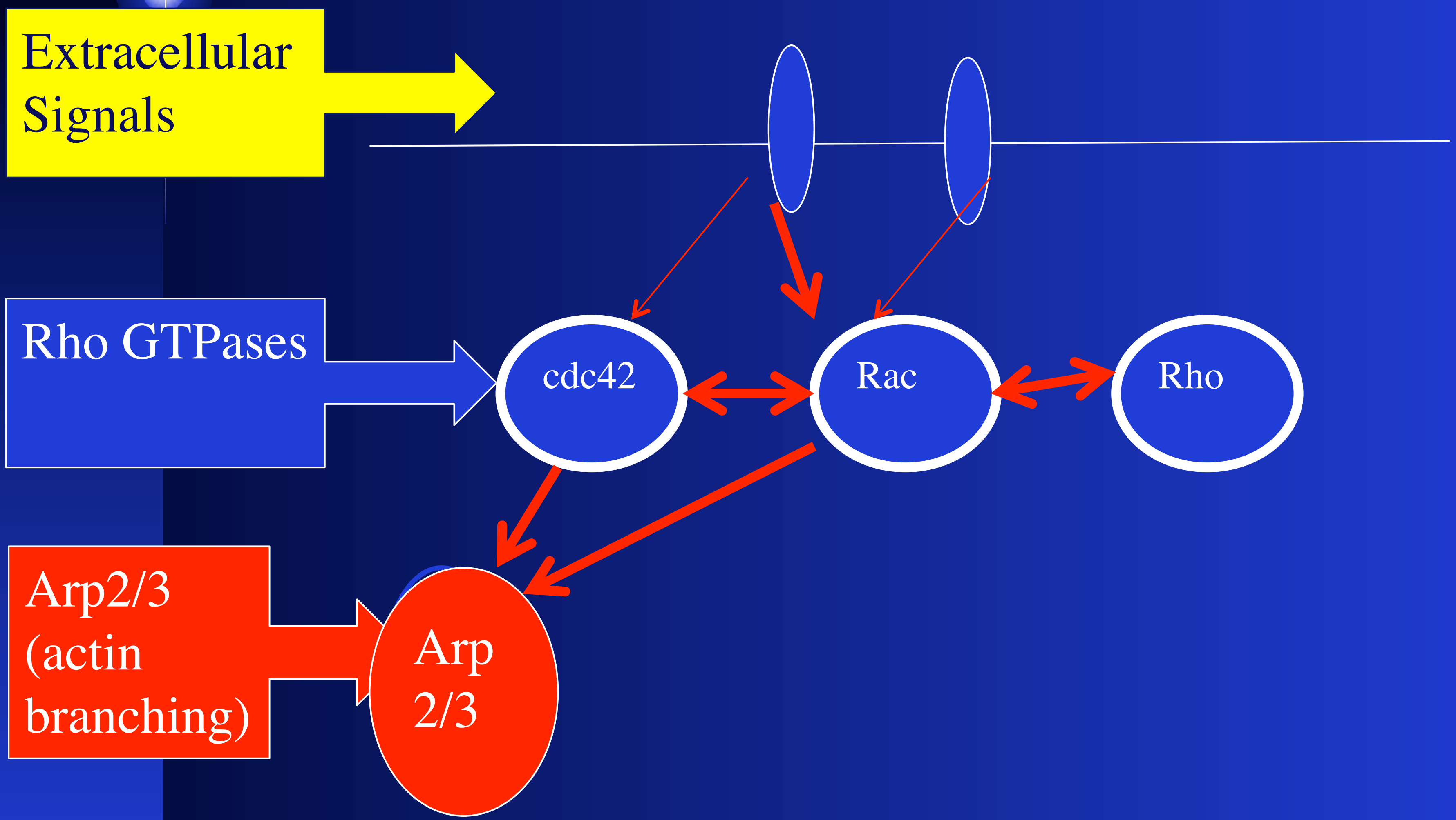
Figure showing how stimuli lead to Arp2/3 activation, actin dendritic nucleation, and recycling of actin..

Removed for copyright reasons.

See original article:

Pollard (2003) **The cytoskeleton, cellular motility and the reductionist agenda** *Nature* **422**: 741-745

GTPAses signal to Arp2/3



Look at simple models that allow for new actin filament barbed ends (filament tips)

Simplest branching model

$$\frac{dn}{dt} = \phi F - \kappa n,$$

$$\frac{dc}{dt} = -k_f c n + \delta F,$$

$$\frac{dF}{dt} = k_f c n - \delta F.$$

Total amount $A = c + F$ is constant

(eliminate F or c)

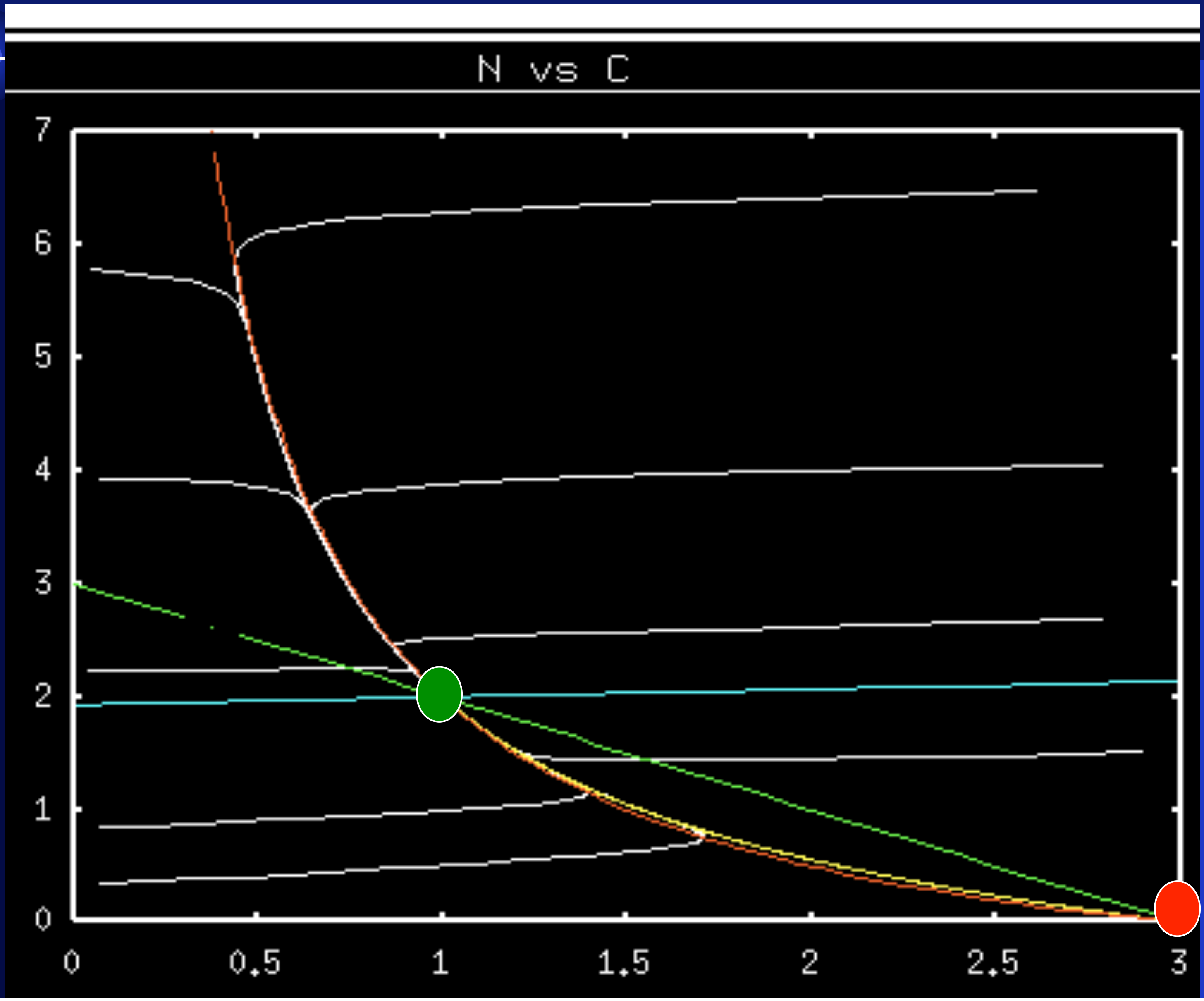
Tips and monomer

$$\frac{dn}{dt} = \phi F - \kappa n,$$

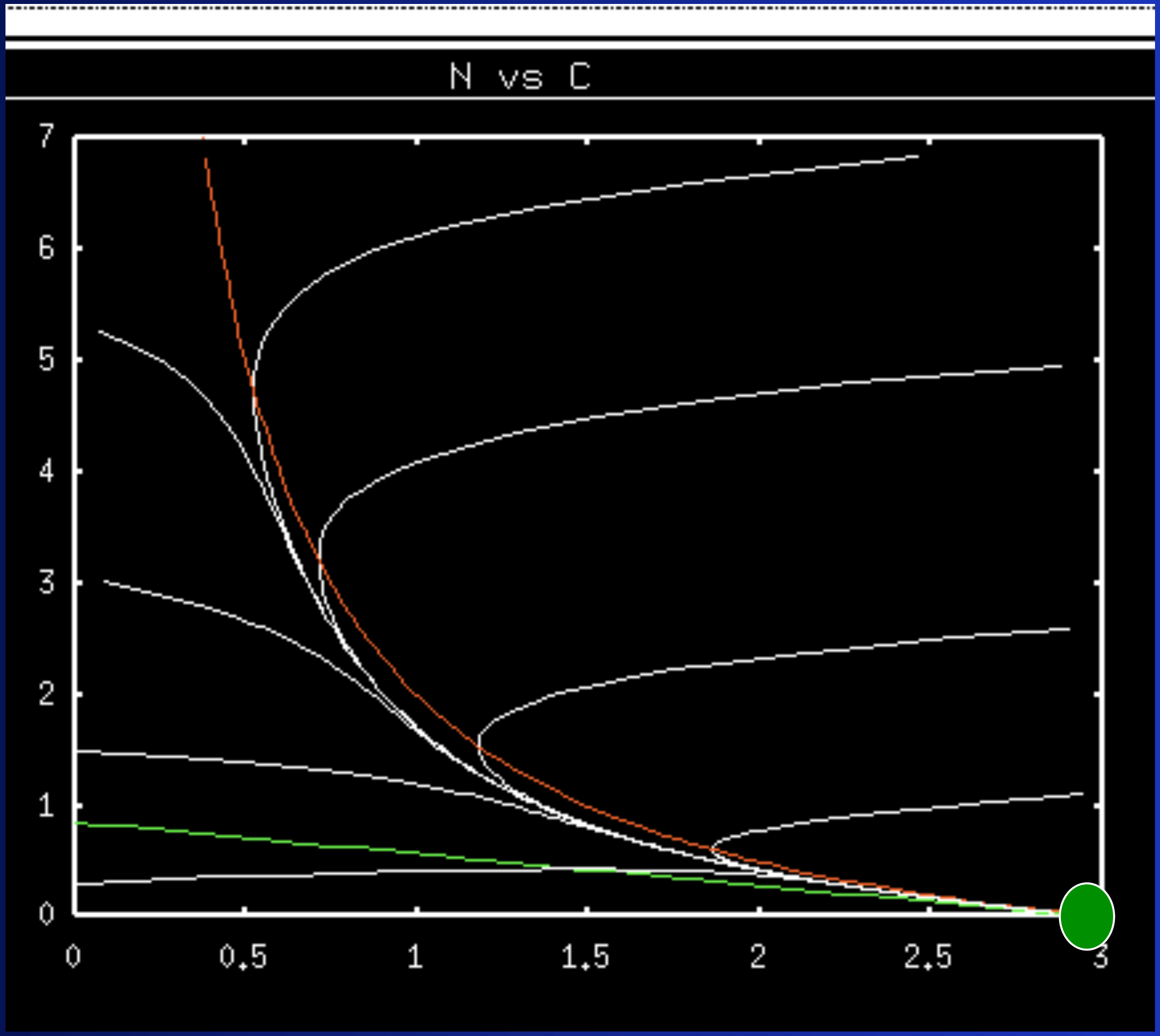
$$\frac{dc}{dt} = -k_f c n + \delta F.$$

Substitute $F = A - c$

Low capping rate: two steady states

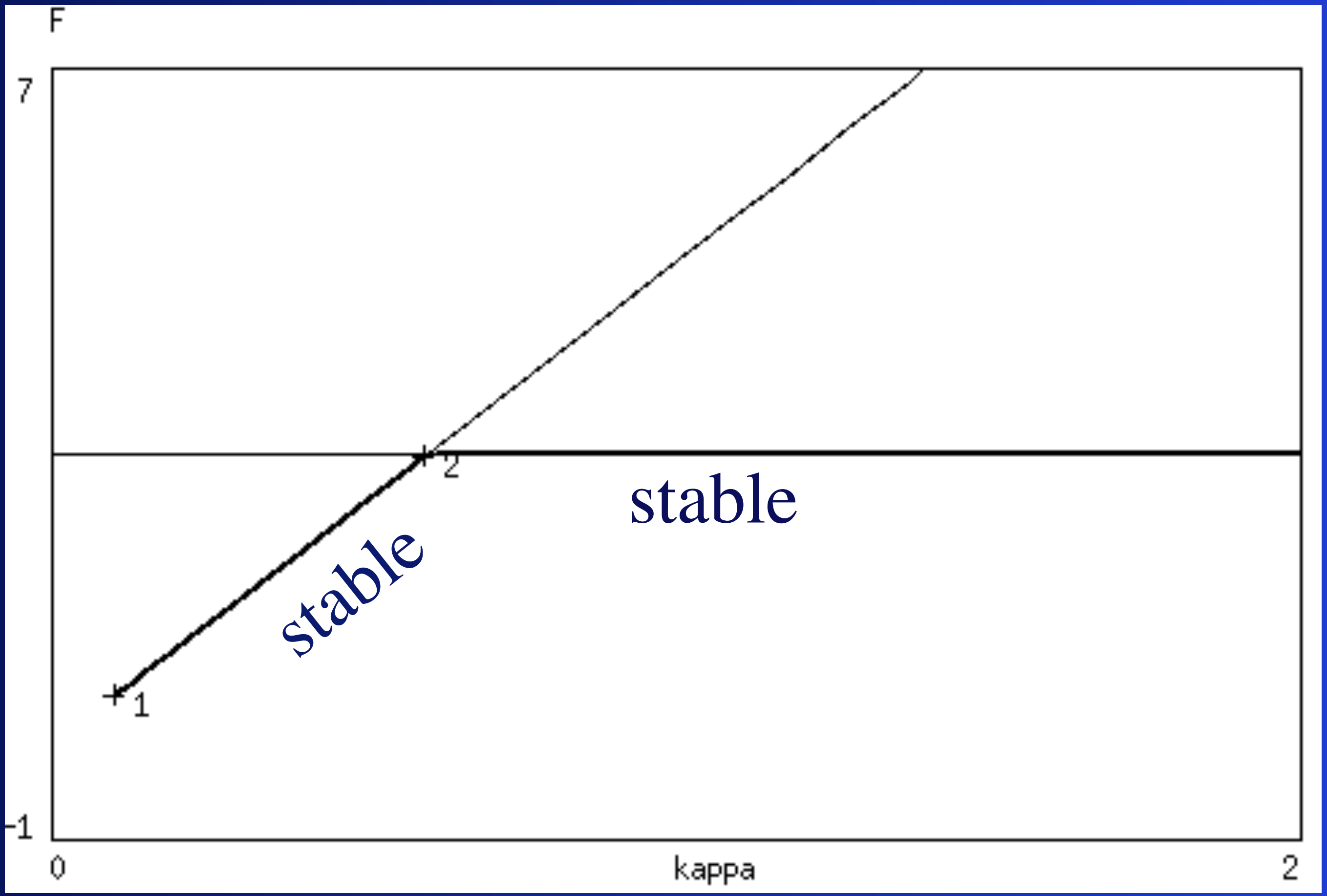


High capping rate: one steady state



Transcritical bifurcation at ϰ=0.6

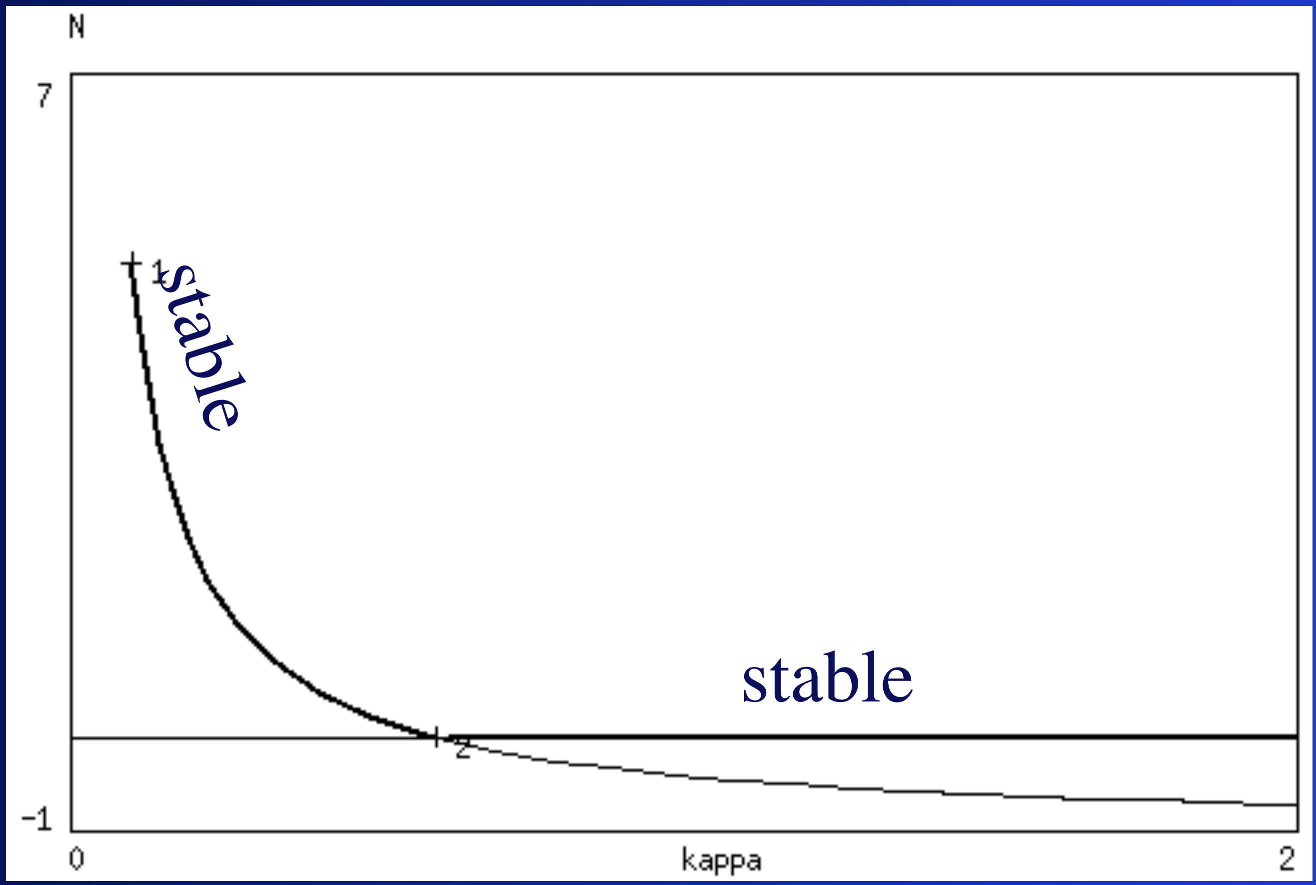
Polymer



Capping rate

Tips no longer available beyond some level of capping

Number of (uncapped) ends



Capping rate

Simulation file

```
# TipsandCap.ode
#
# Simulation for formation of new filament tips
#
#
dc/dt=-kf*c*n +delta*(A-c)
dn/dt=phi*(A-c)-kappa*n
#
aux F=A-c
#dF/dt=kf*c*n -kr*(A-c)
param kf=1, delta=1, kappa=0.1, phi=0.2, A=3
init c=2.9, n=1
@
total=20,xp=c,yp=n,xlo=0,xhi=3,ylo=0,yhi=7
done
```

Actin branching and capping

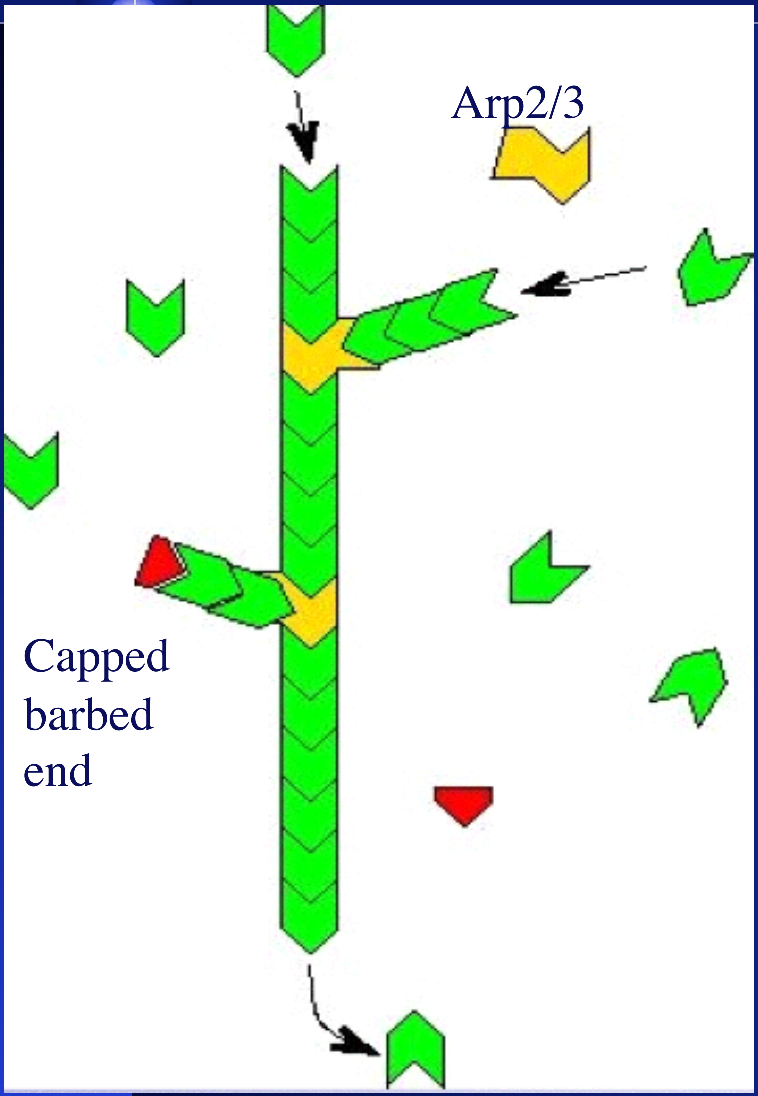
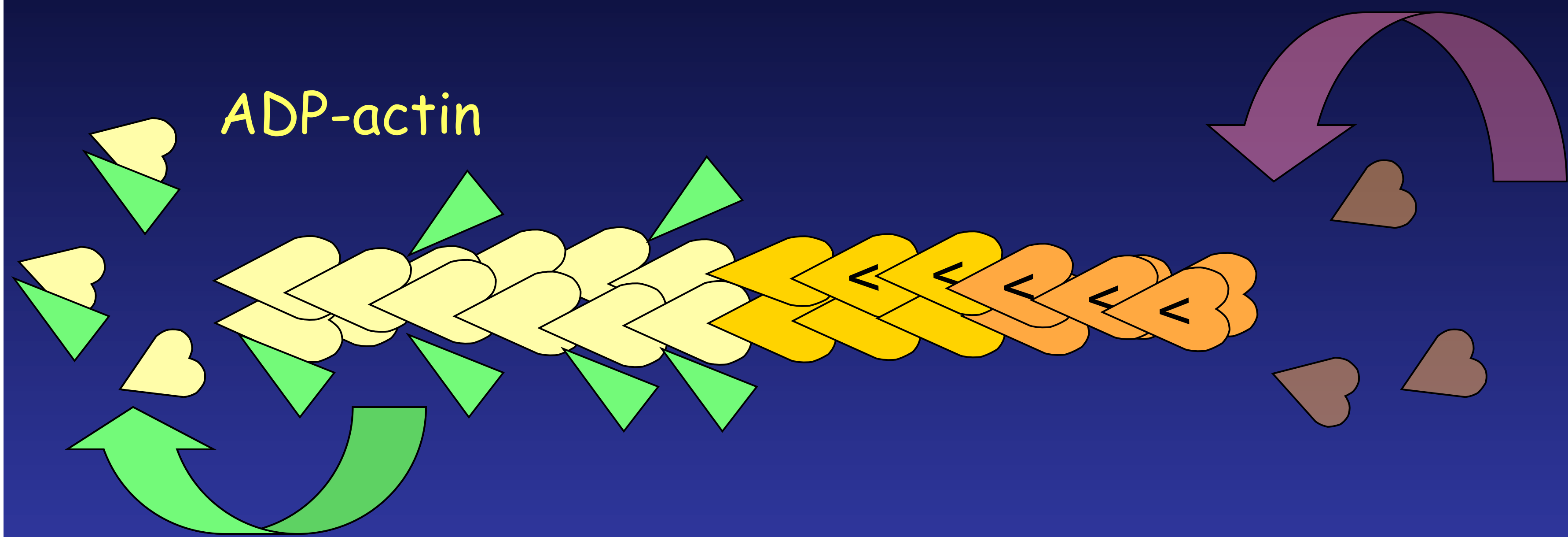
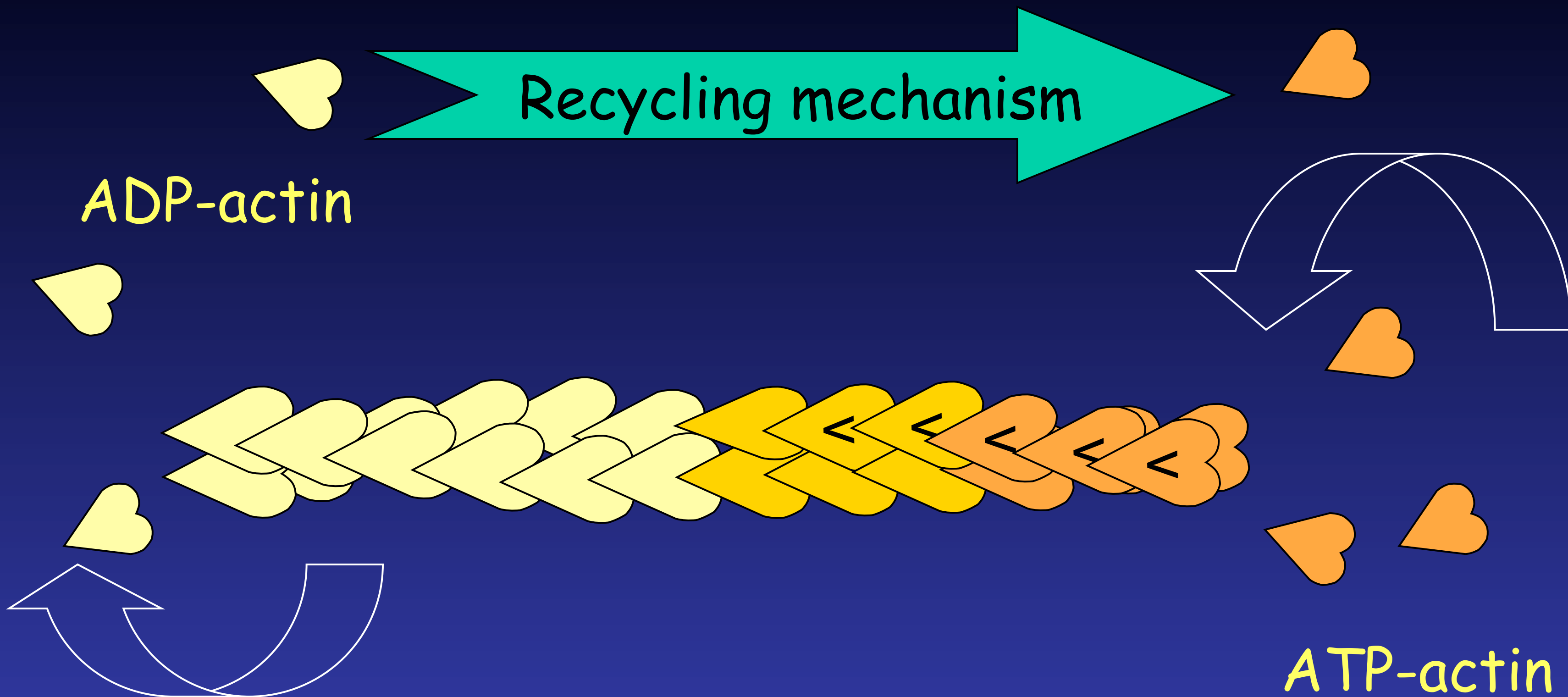


Fig courtesy: A.T. Dawes

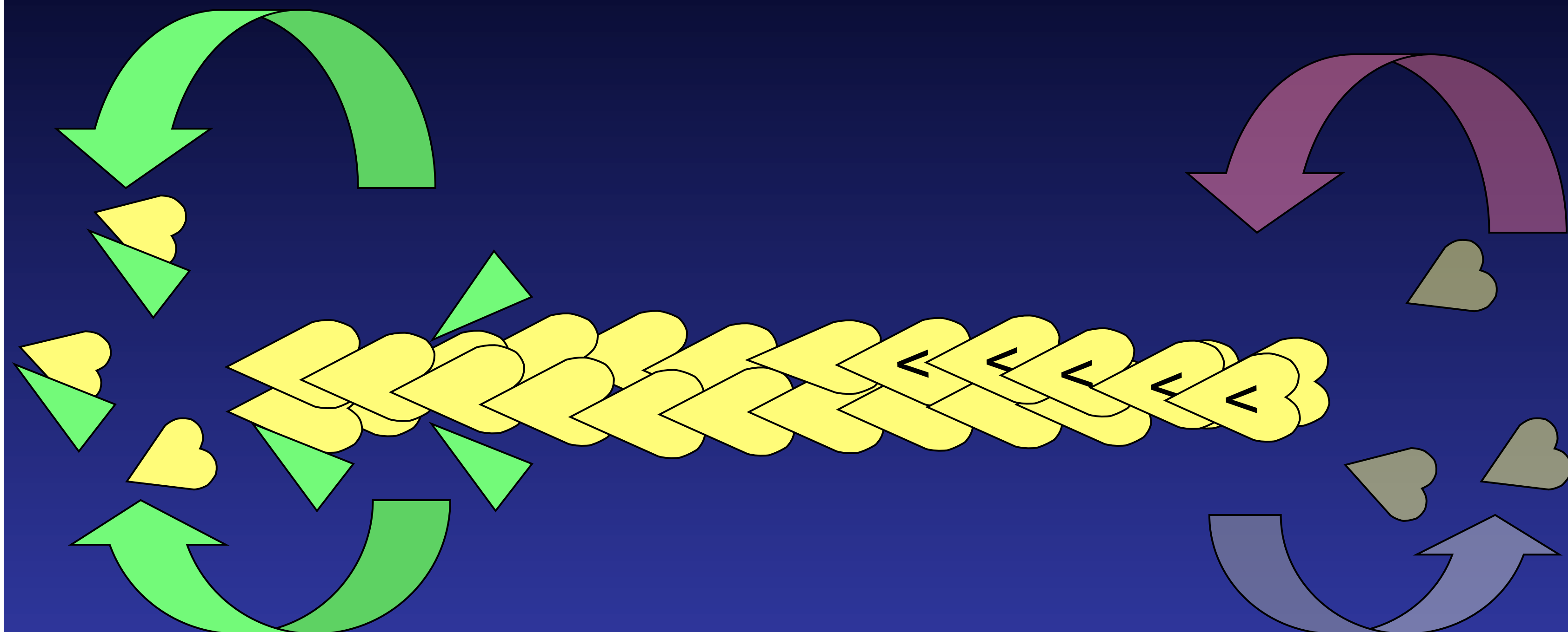
- Polar filaments polymerize fastest at their “barbed” ends, slower kinetics at the “pointed ends”
- Barbed ends regulated by capping, branching



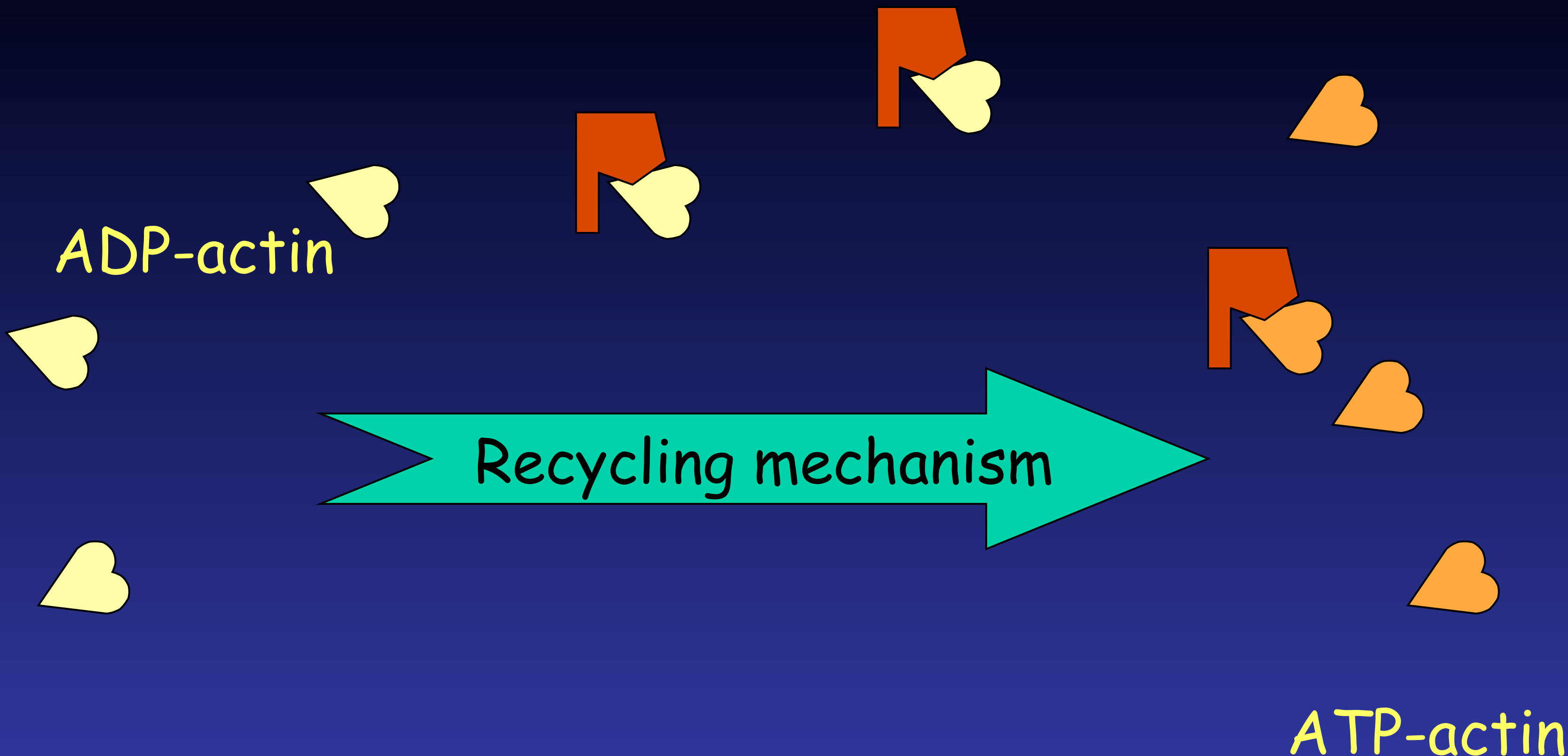
Cutting and fragmenting occurs fastest at the older (ADP-actin) parts of an actin filament



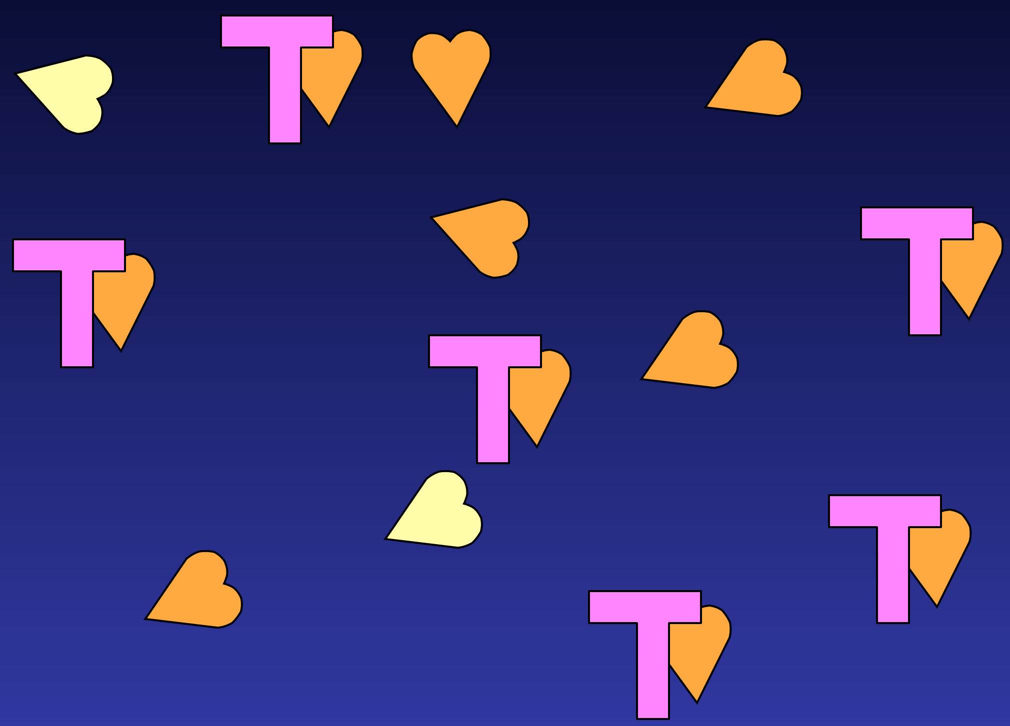
There are mechanisms for converting “spent” ADP-actin monomers into their active form



Proteins such as cofilin and gelsolin break up filaments



Action of profilin



Thymosin sequesters actin monomers

(to control the rate of polymerization)

Actin monomers available in the cell

ATP – actin fastest to polymerize

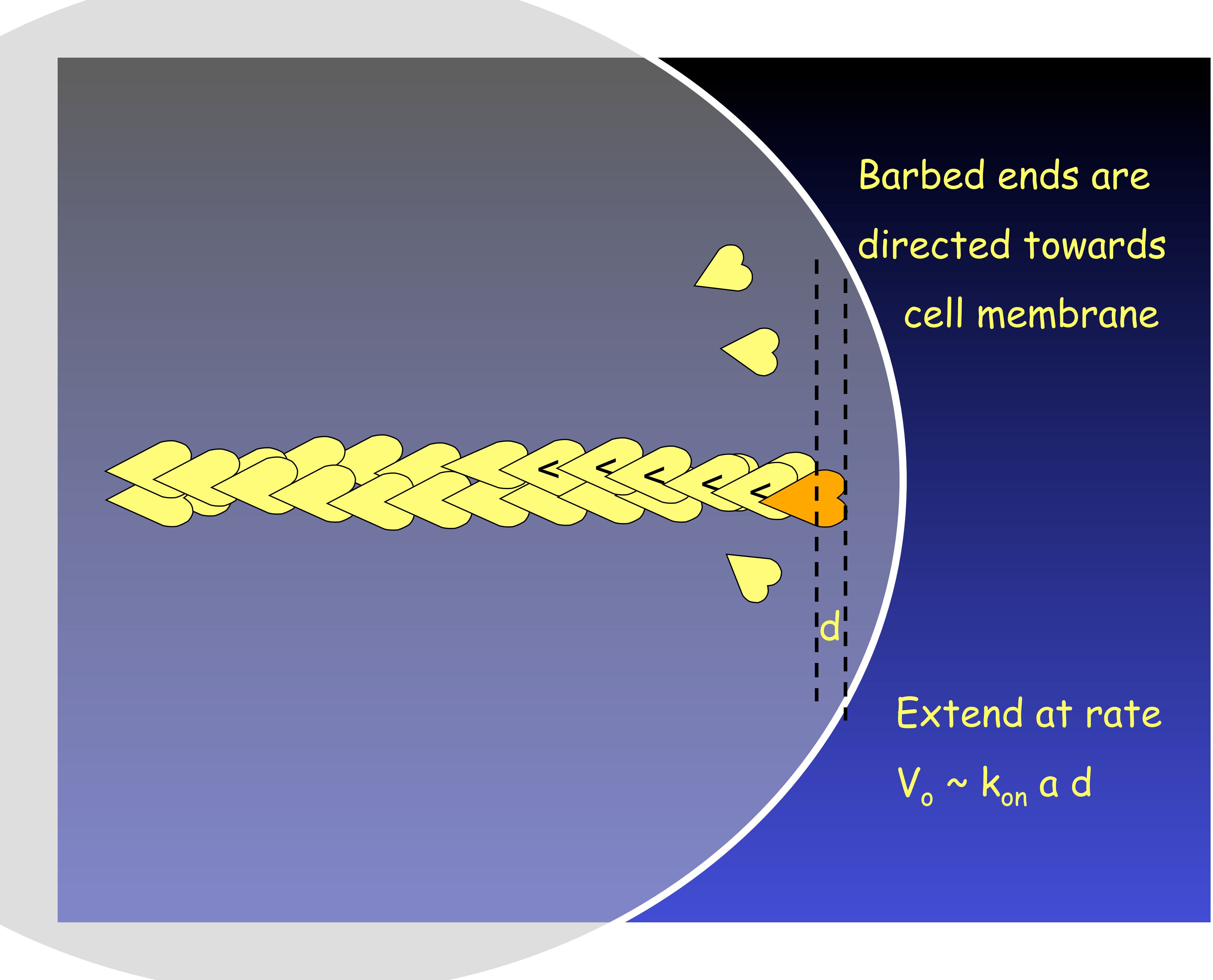
Buffered at roughly constant level inside cell. Profilin helps to recycle, Thymosin stores it in “sequestered” pool.

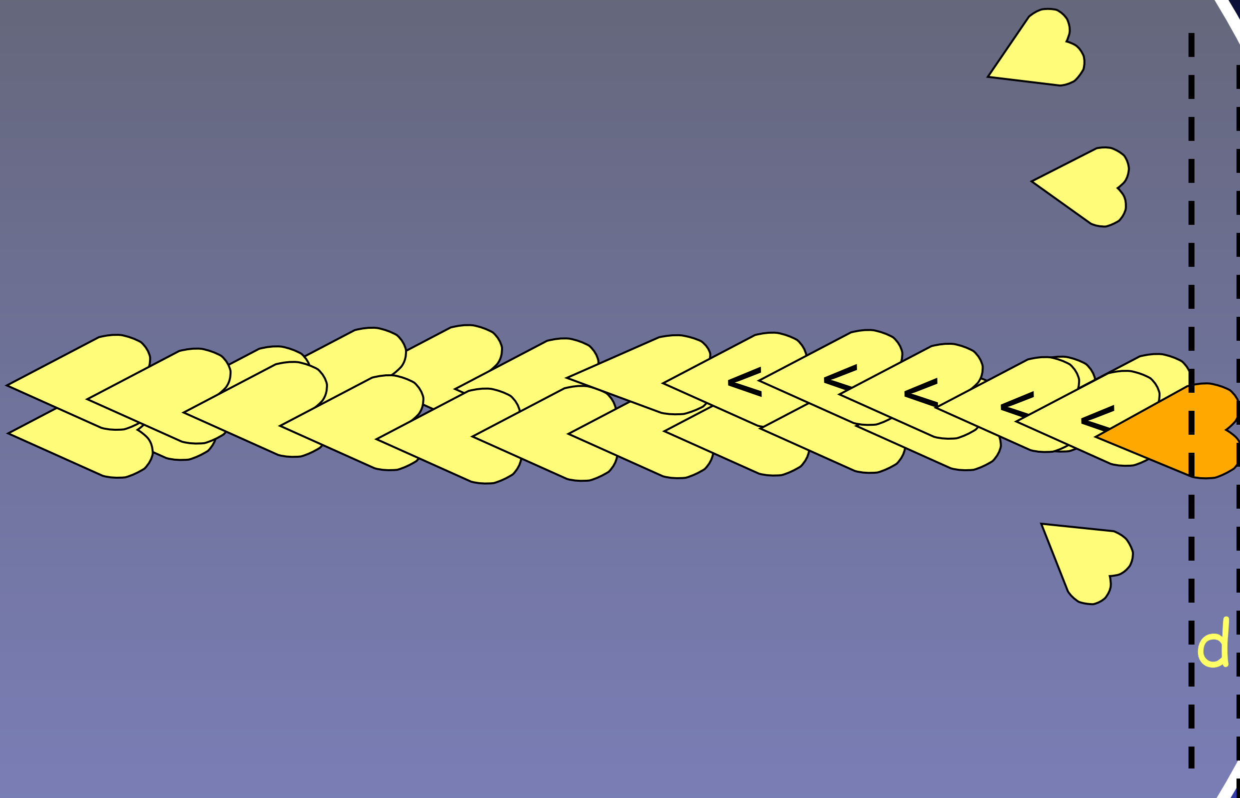
Barbed ends that are uncapped would grow at some constant rate

Regulation by capping and nucleation of those ends, less by monomer concentration.

Where in the cell is polymerization most important?

- At edge of cell .. To cause protrusion against load force..
- To notice: thermal ratchet… polymerization against a load force
- One question: can monomers get to the front edge fast enough by diffusion to account for protrusion speed?

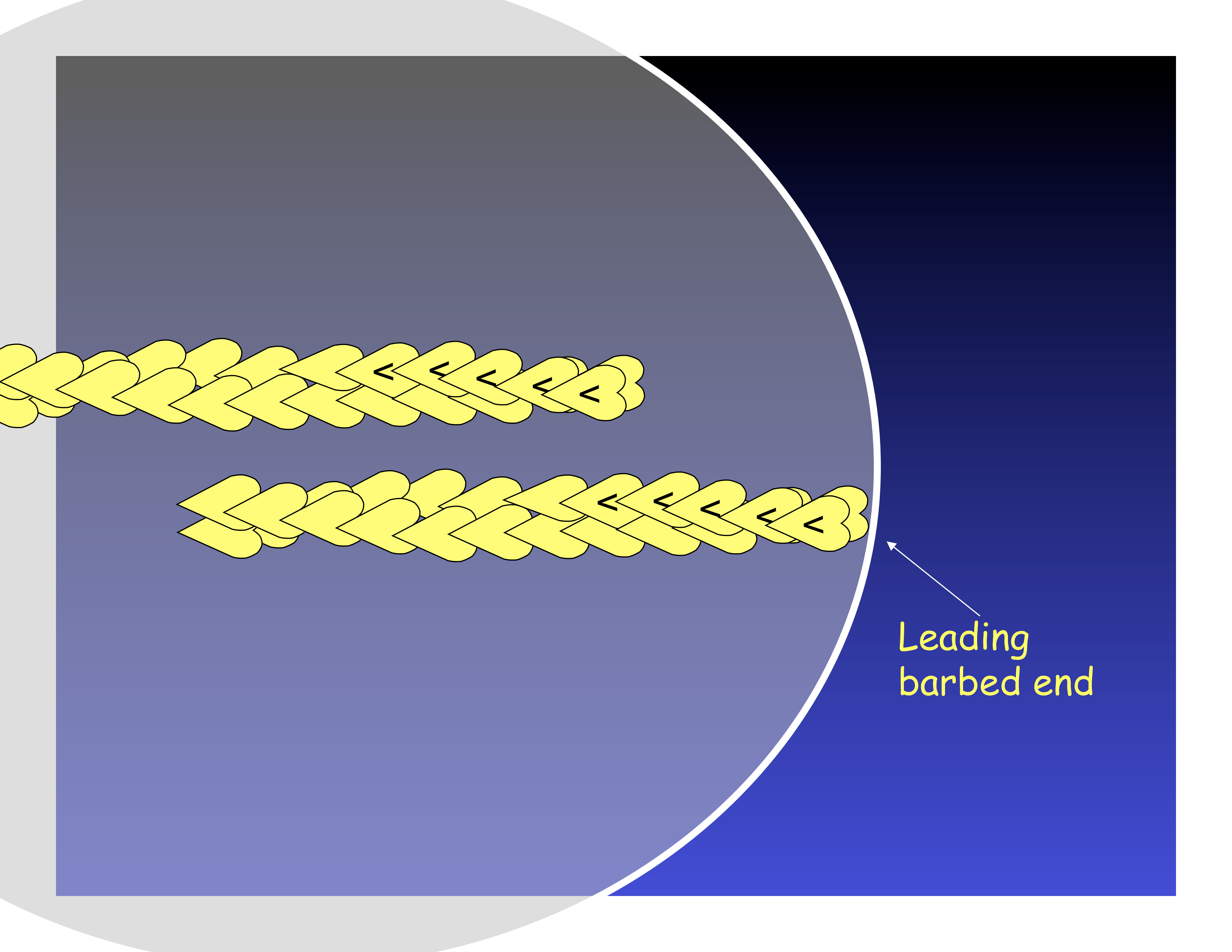

Barbed ends are
directed towards
cell membrane
d
Extend at rate
$V_o \sim k_{on}\, a\, d$



$V_o \sim k_{on}\ a\ d$

a = actin conc at membrane

d = size increment of one monomer


Leading
barbed end

Coupling biochemistry and mechanics of motion

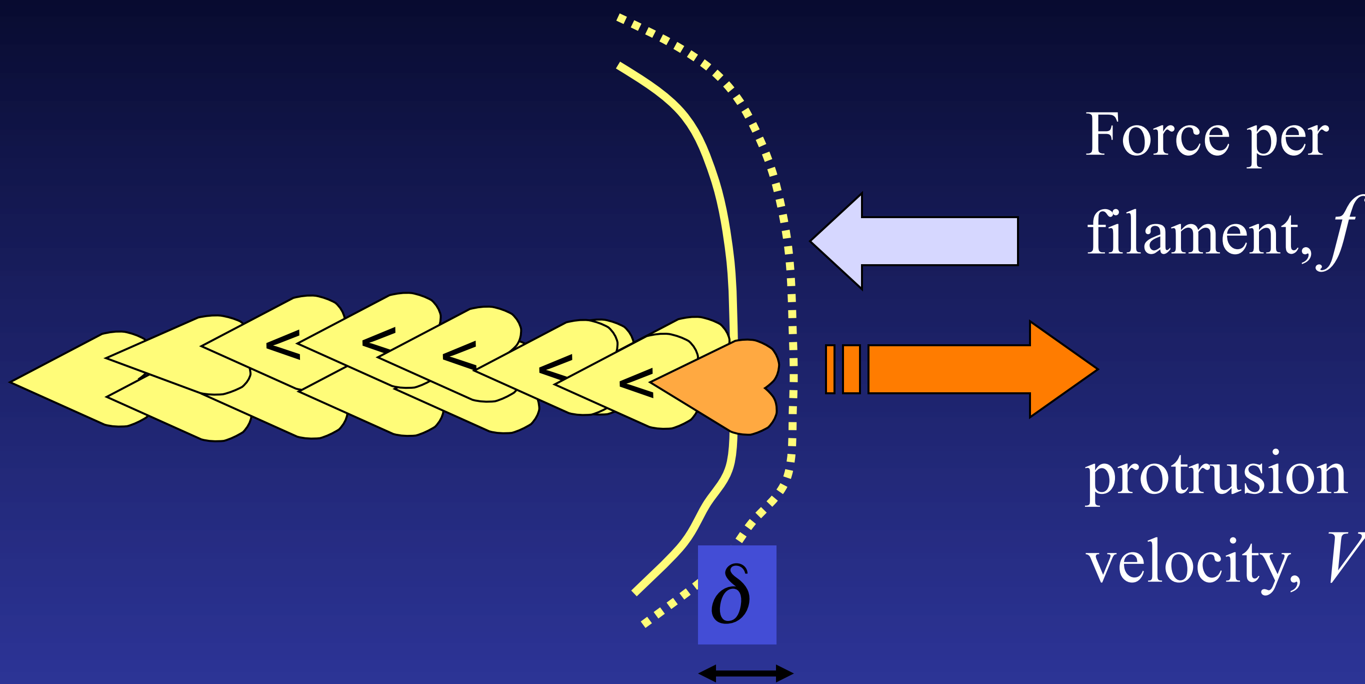
Mogilner & Oster (1996) Biophys J, 71: 3030-3045

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

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

Thermal energy

Speed of motion of one filament barbed end

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

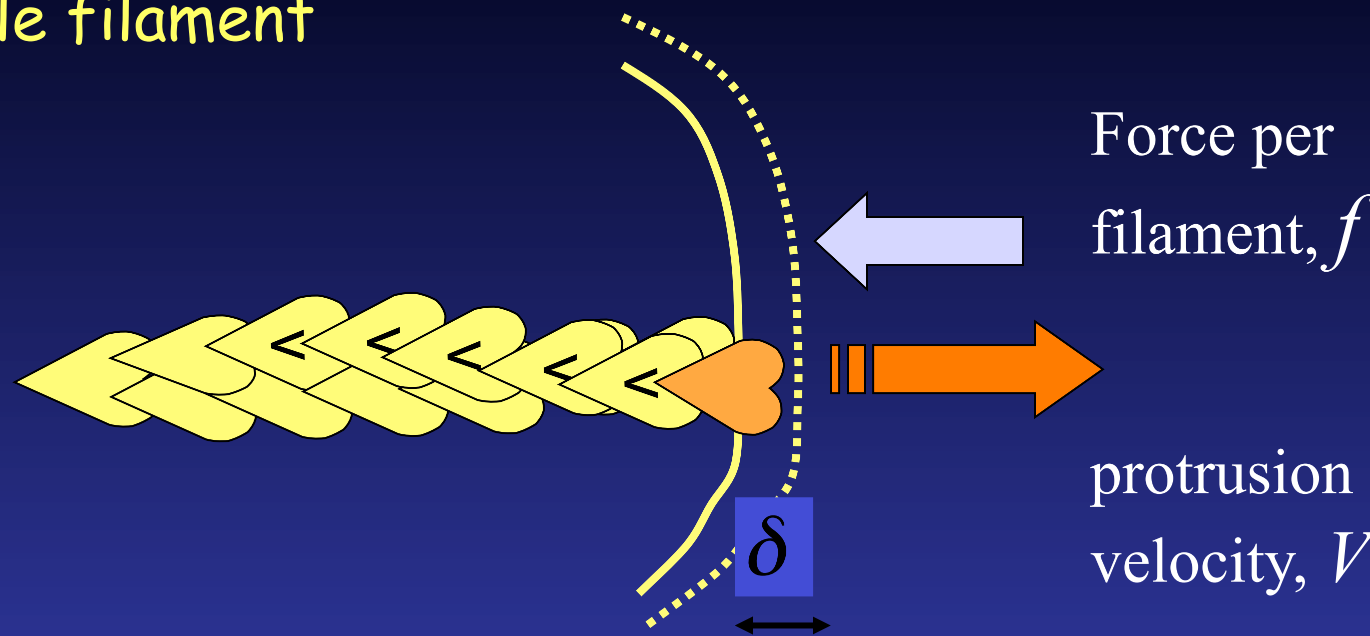
on rate

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

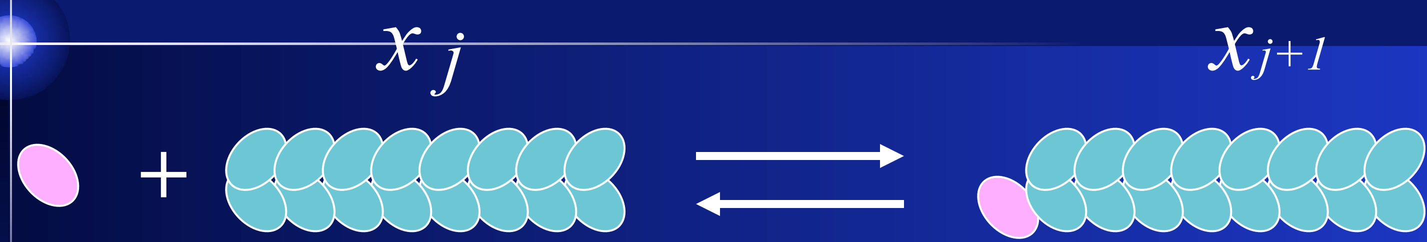
Thermal energy

Polymer size distribution

Filament size distribution

It is very common in math-biology to consider size classes and formulate equations for the dynamics of size distributions (or age distributions, or distribution of some similar property).

Number of filaments of length *j* :



$$\frac{dx_j(t)}{dt} = \underbrace{k^+ a x_{j-1}}_{} - \underbrace{(k^- + a k^+) x_j}_{} + \underbrace{k^- x_{j+1}}_{}$$

Growth of shorter filament

Monomer loss or gain

Shrinking of longer filament

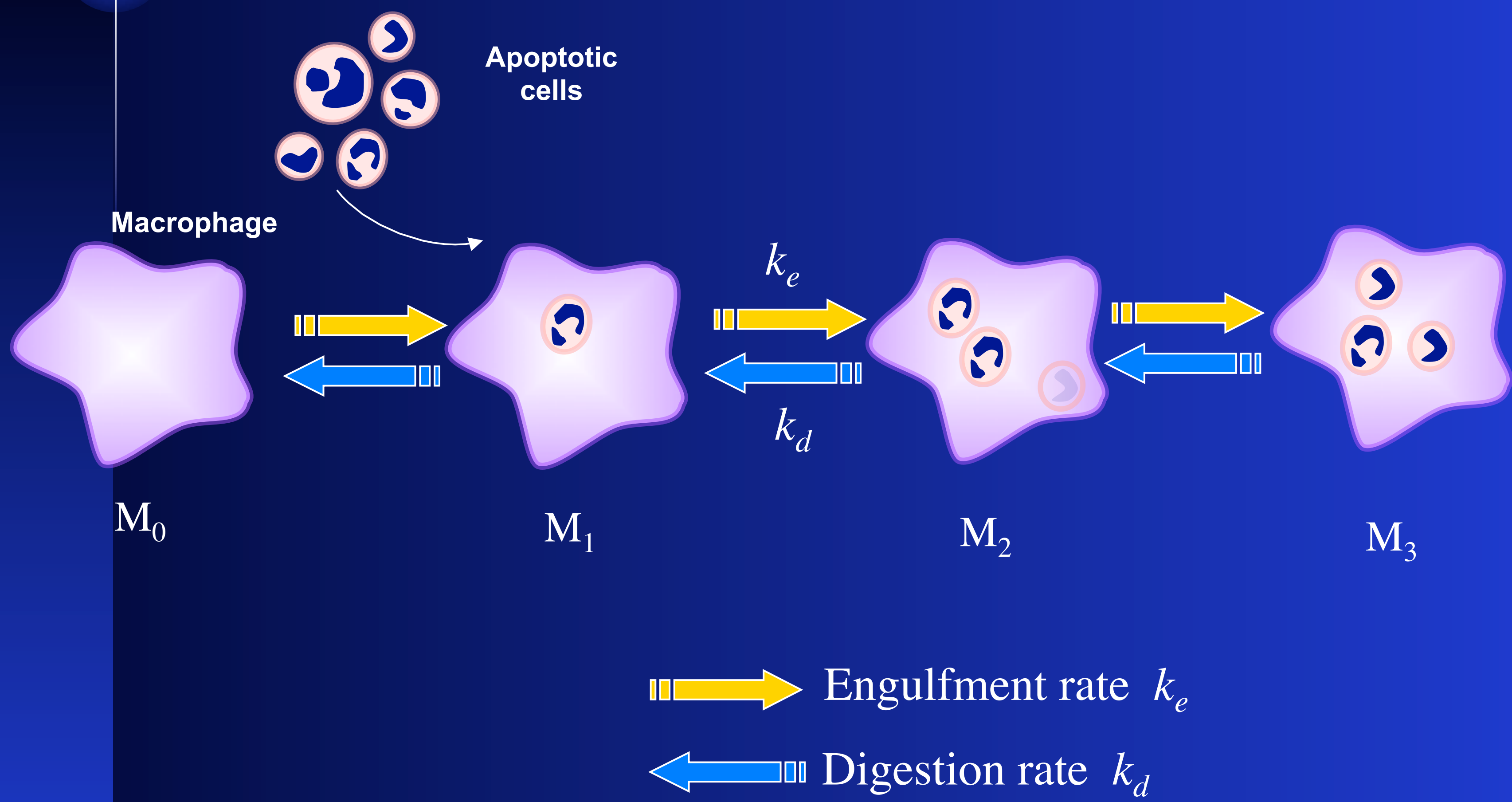
Steady state size distribution for constant pool of monomer

$$\frac{dx_j(t)}{dt} = k^+ a x_{j-1} - (k^- + a k^+) x_j + k^- x_{j+1}$$

Find the steady state size distribution (assume that a, $k+$, $k-$ are constant.)

Express this in terms of $r = a\, k+/k-$

Other applications of same idea



Next: more about polymer size distributions