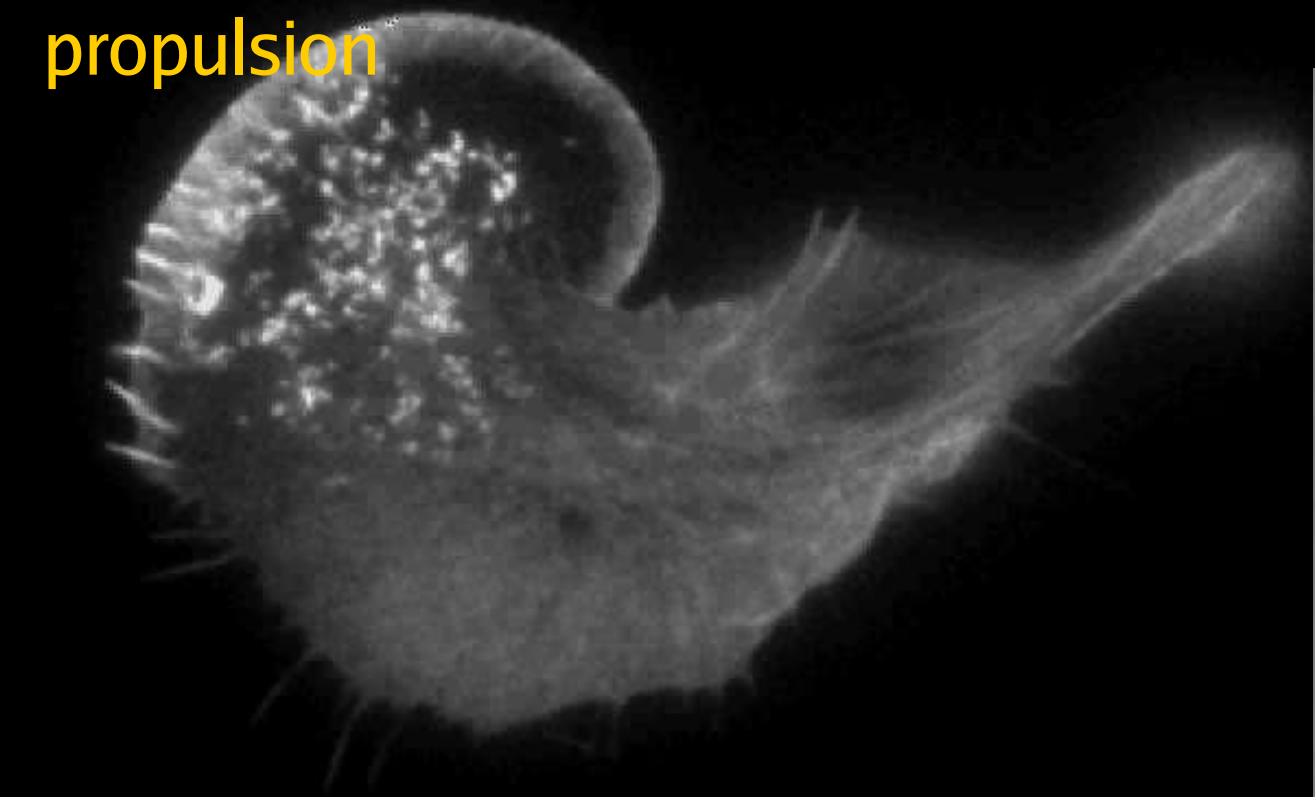
Actin-based Motility

Actin-GFP

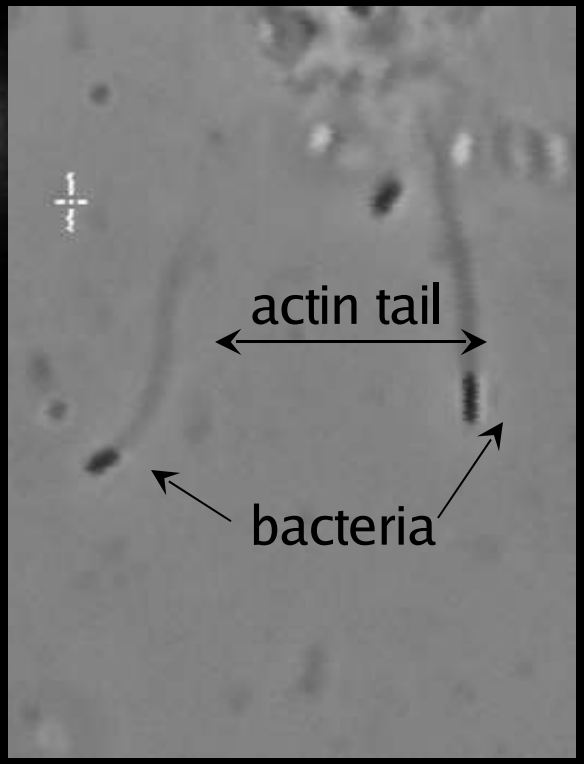
Lamellipodium and filopodium extension

propulsion

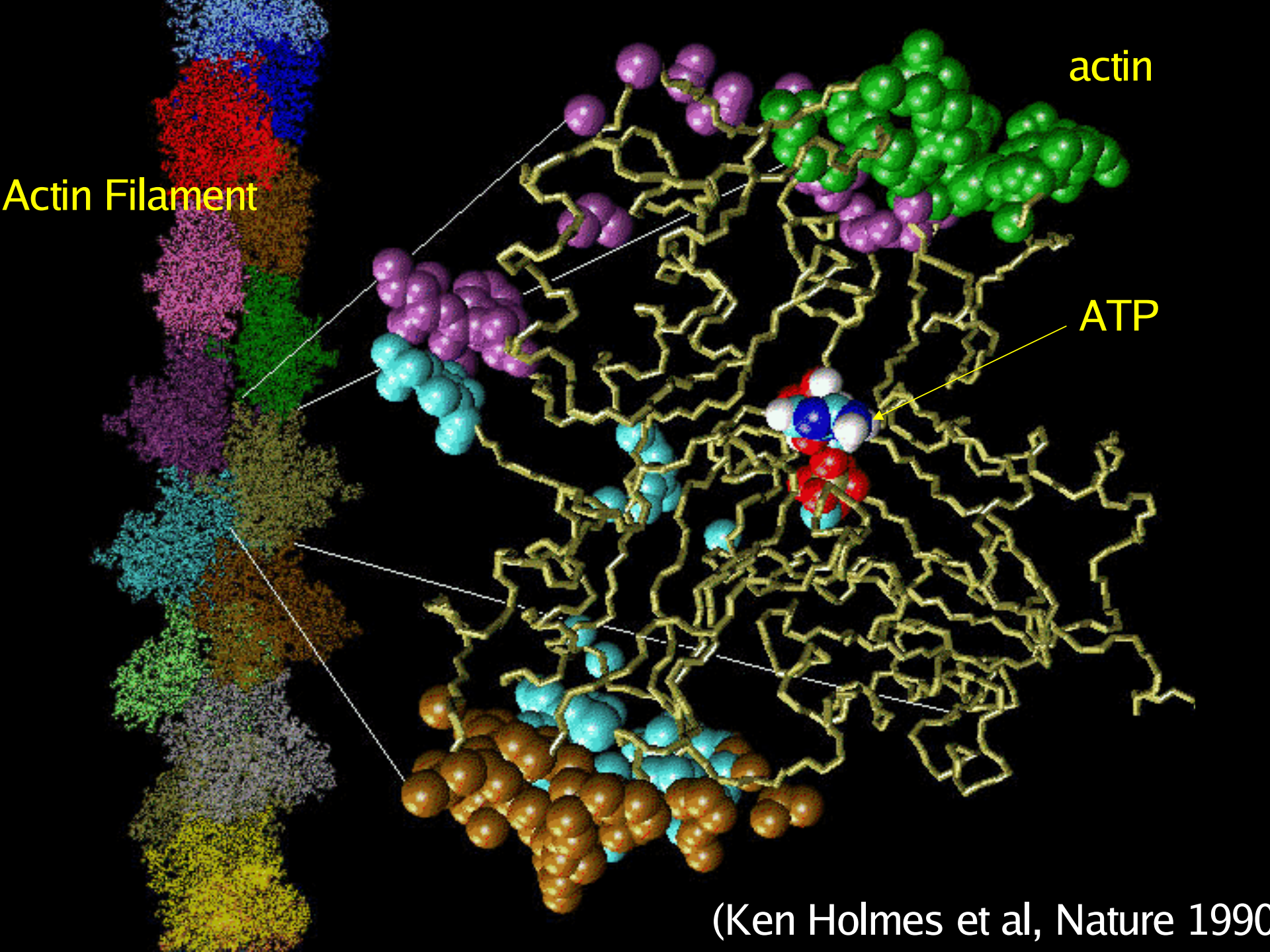


(Vic SMALL)

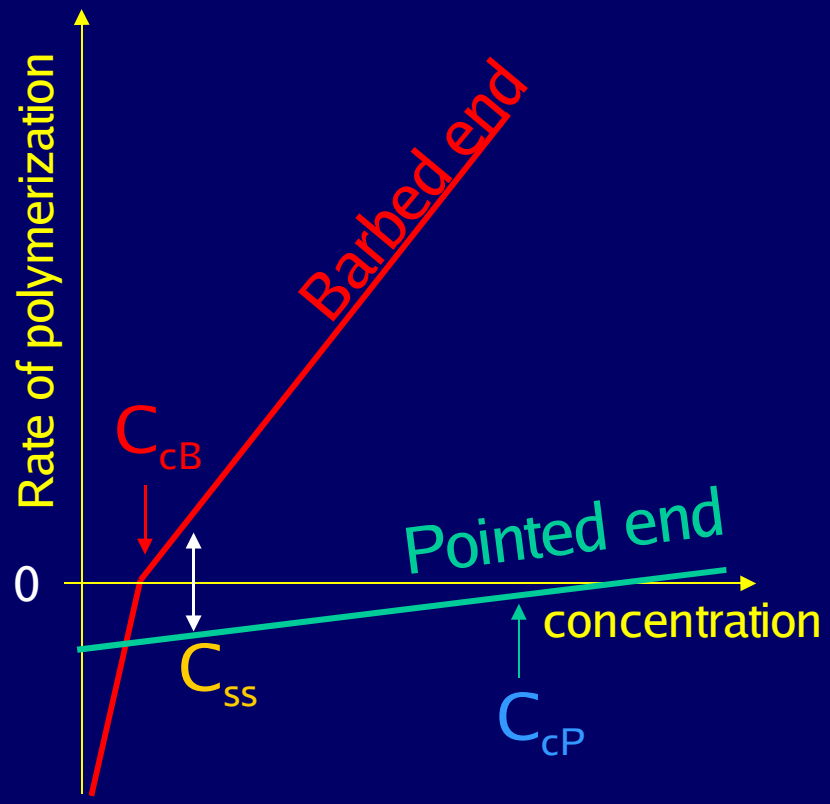
Listeria

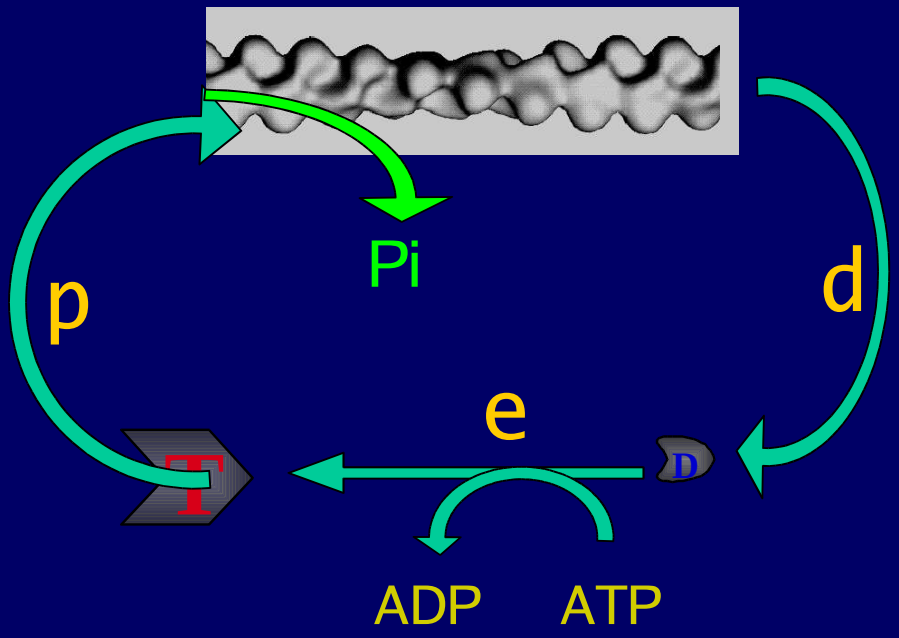


(M-F Carlier et al)


actin
Actin Filament
ATP
(Ken Holmes et al, Nature 1990

Treadmilling

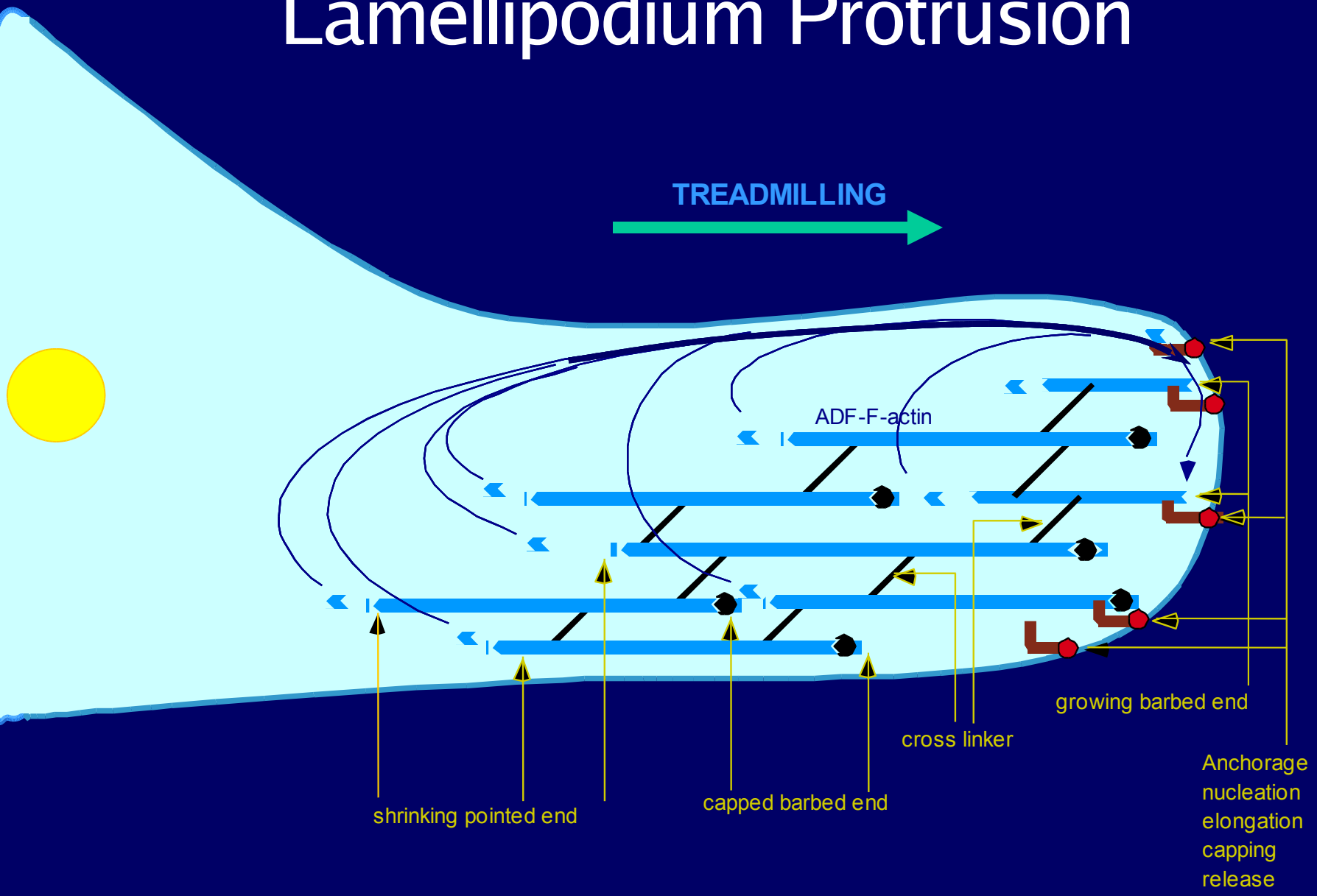




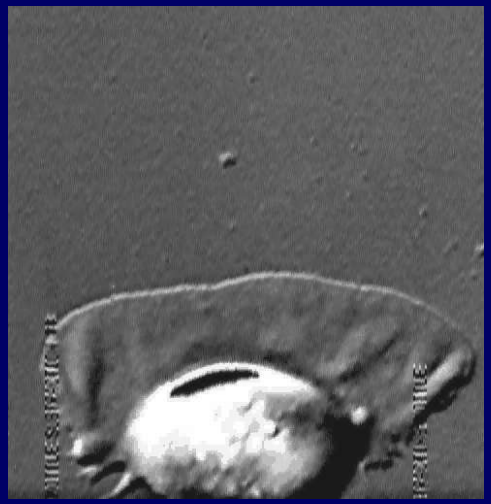
At steady state the filament treadmills :

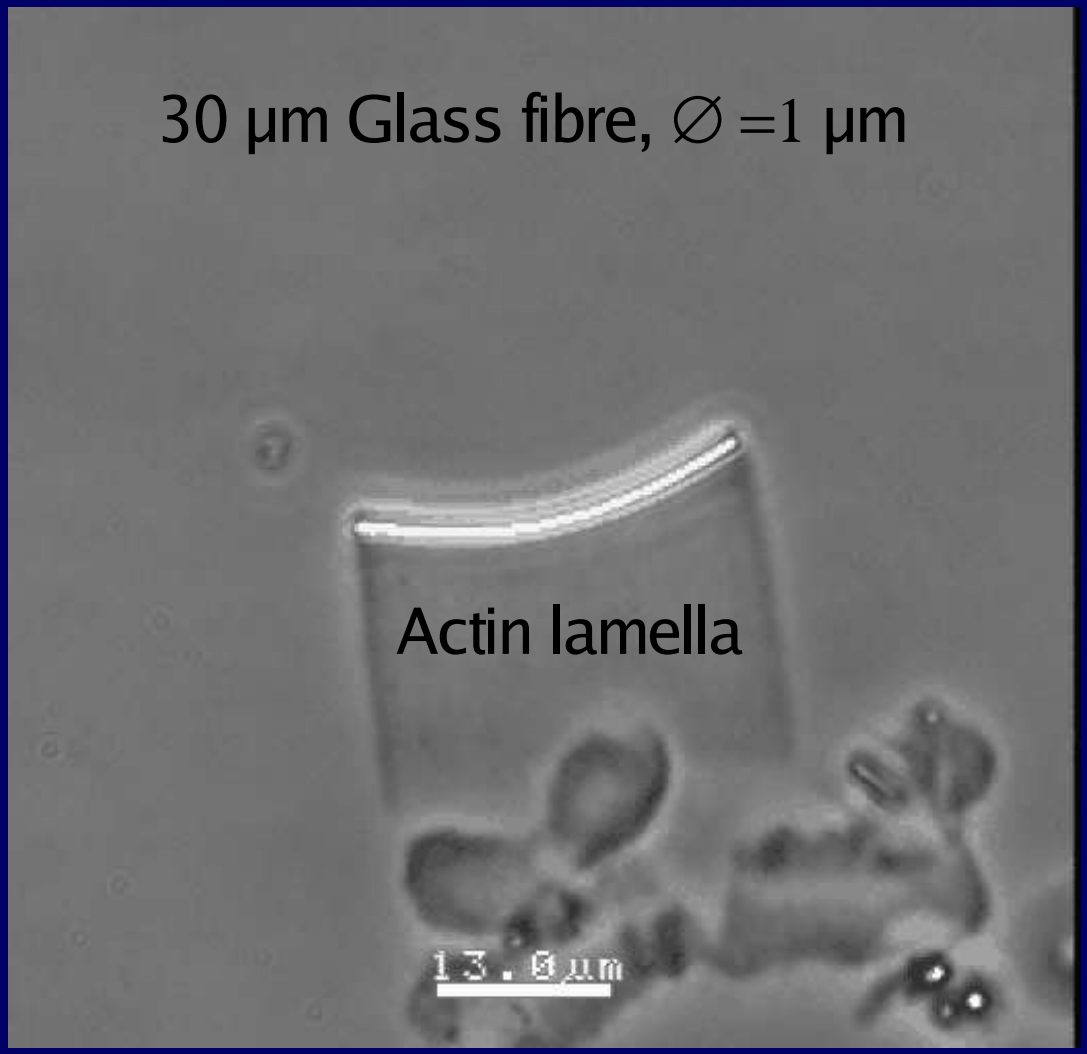
Subunit flux = d = e = p = $k_{+B}.(C_{ss}-C_{cB})$

Lamellipodium Protrusion

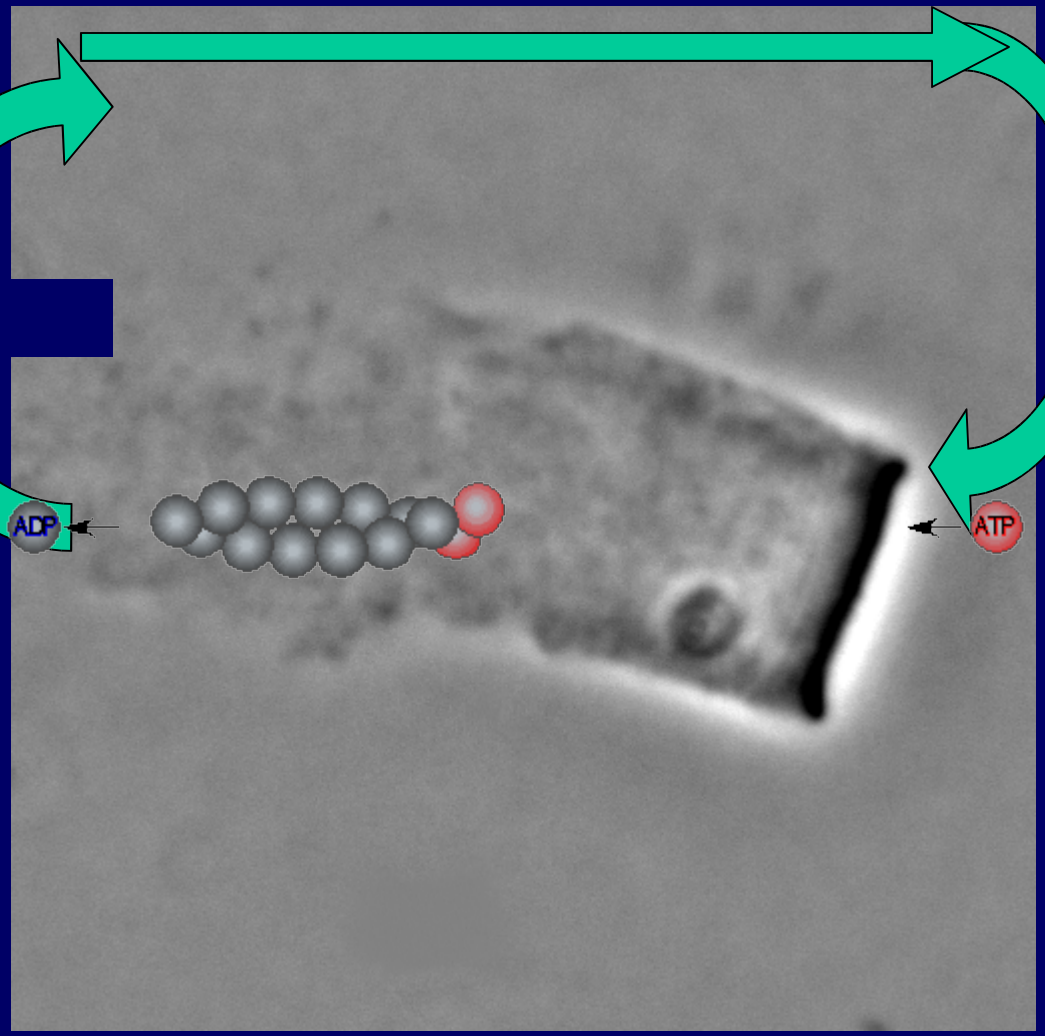


Mimicking lamellipodium with a glass rod ?



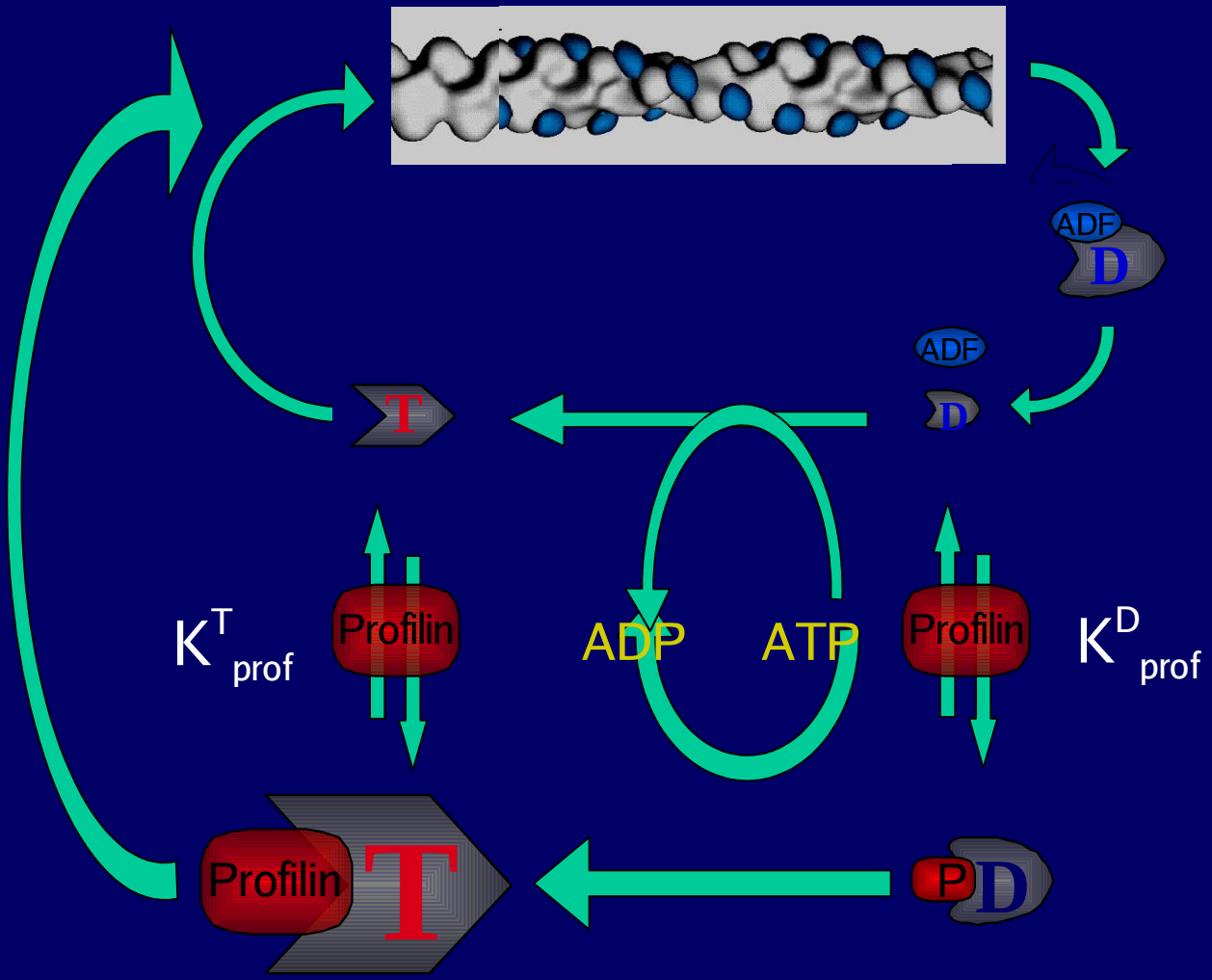


Treadmilling

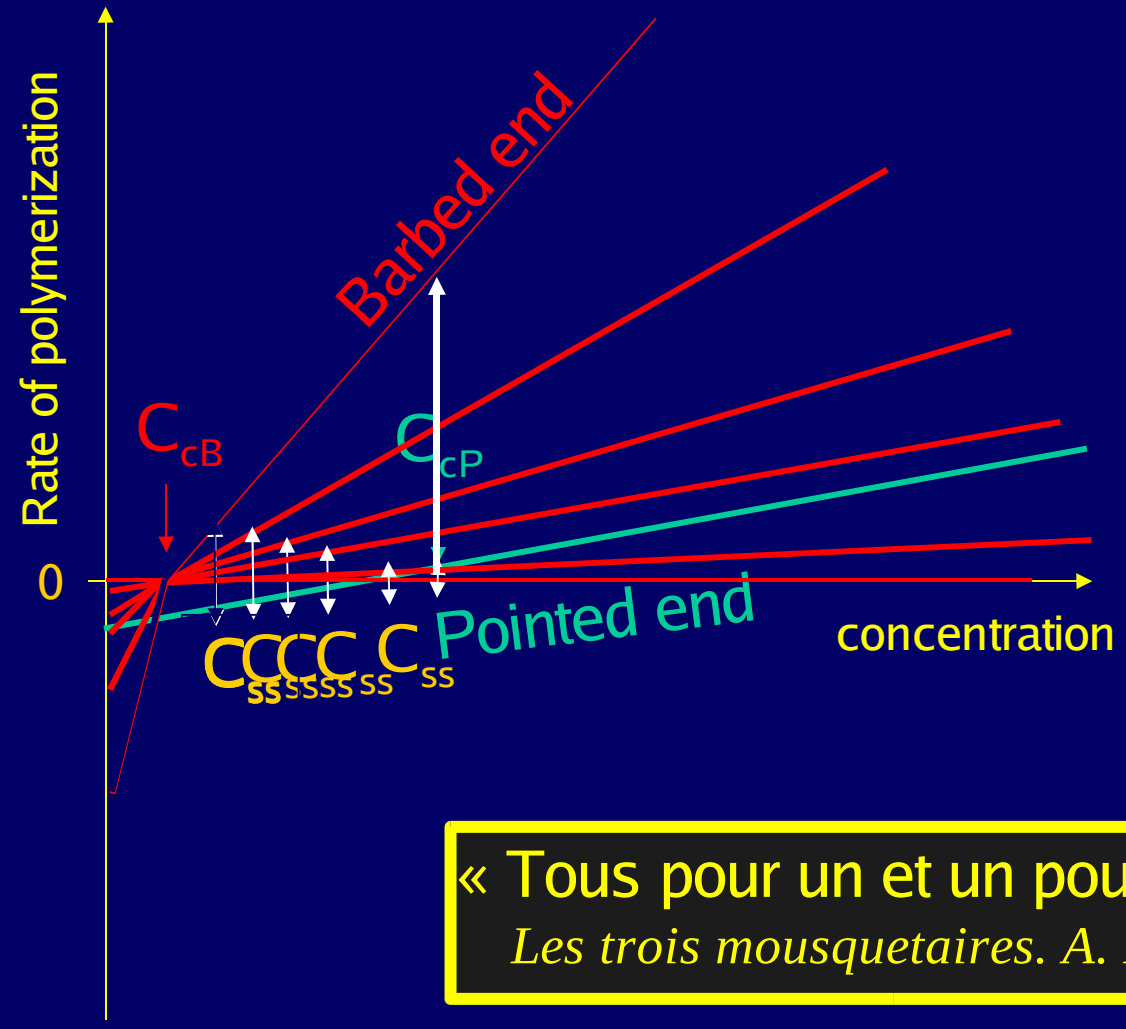


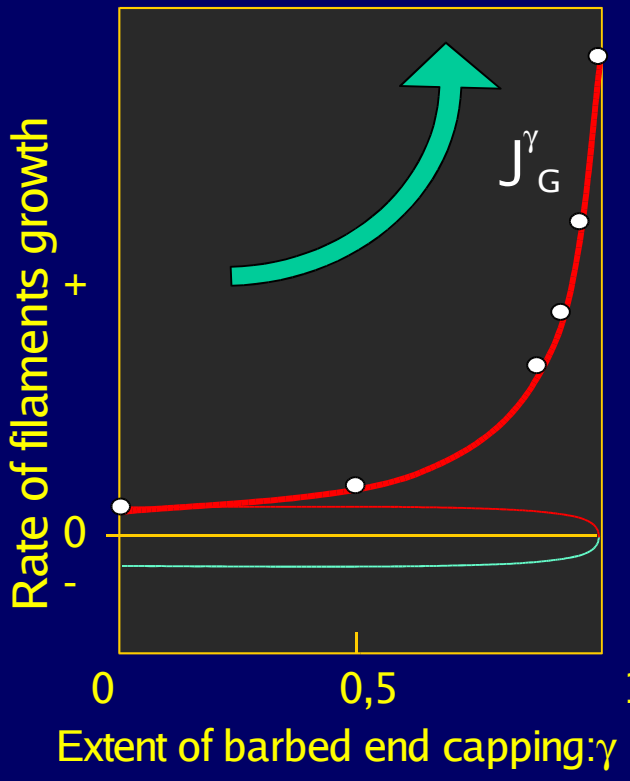
Vitesse = 4µm /min = 66 nm/s = 26 a/fil/s

Synergy between Profilin and ADF



Treadmilling of actin filaments : Effect of capping

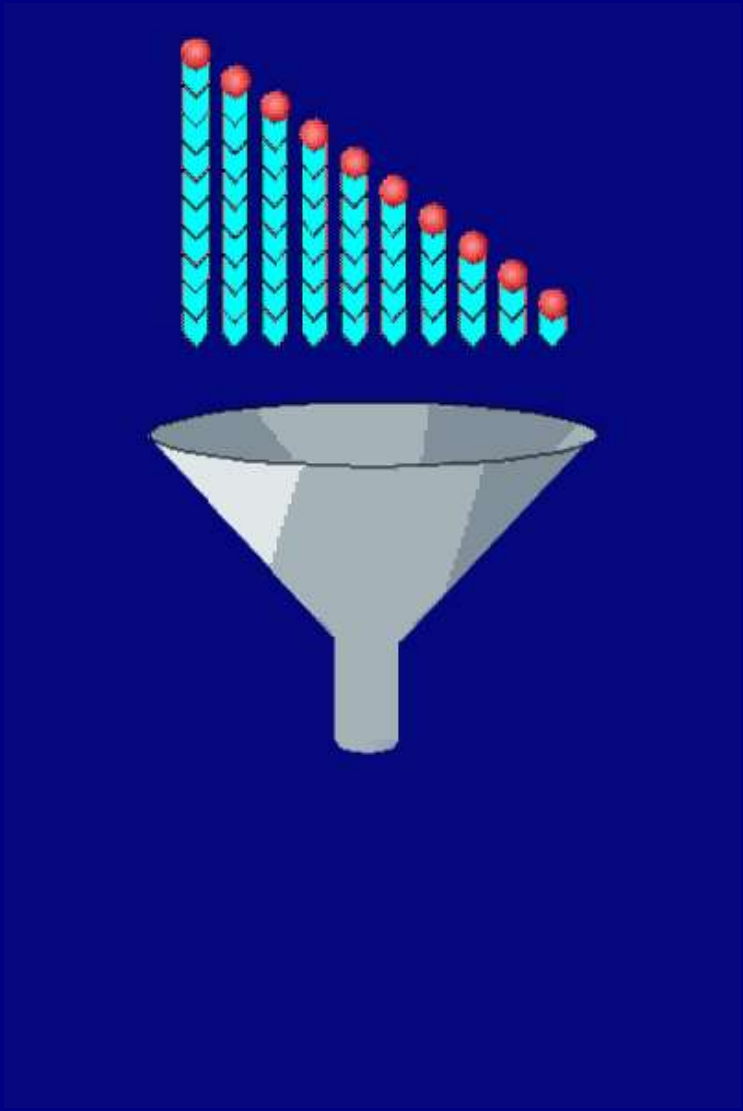




« Tous pour un et un pour tous »

Les trois mousquetaires. A. Dumas

Role of capping proteins in motility: funneling the treadmilling



Slow pointed end disassembly from many capped filaments

Nucleotide exchange

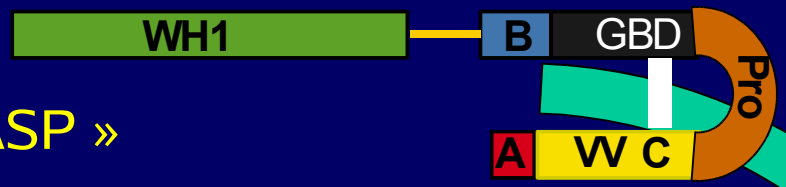
Fast barbed end assembly of few uncapped filaments

and

Capping

WASP family proteins :
activators of Arp2/3 complex

« closed N-WASP »

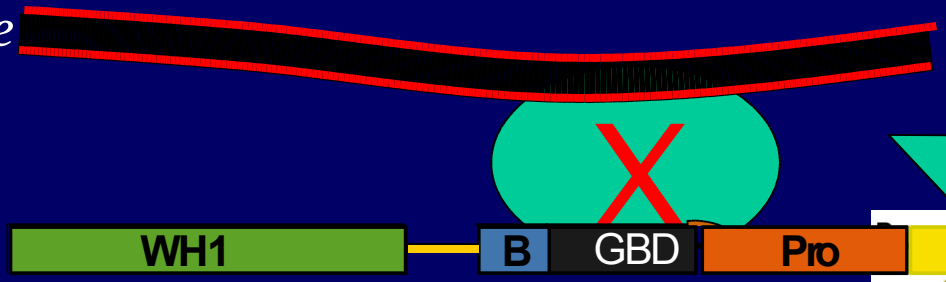


+ X

+ Arp2/3
+G-actin

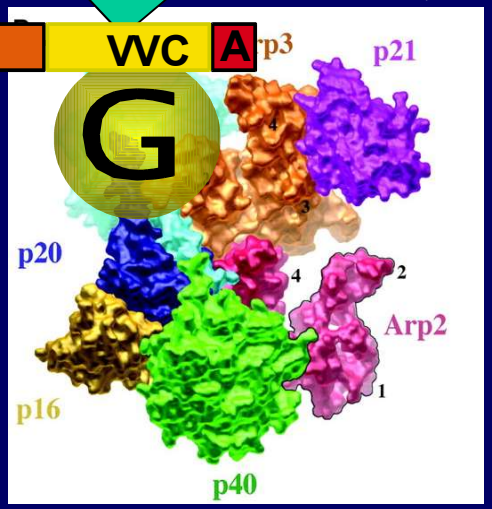
Cdc42
IcsA
TccP

Plasma membrane



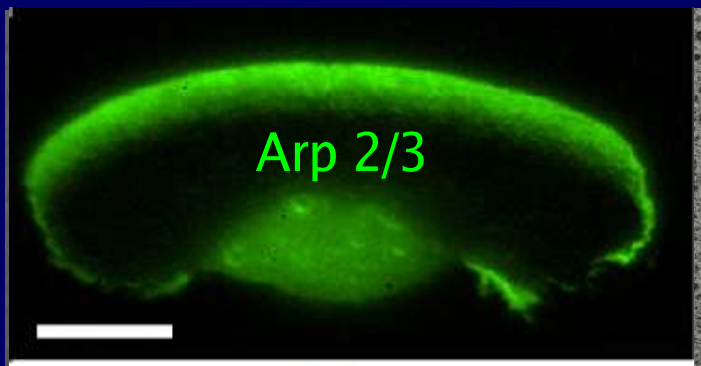
« activated N-WASP »

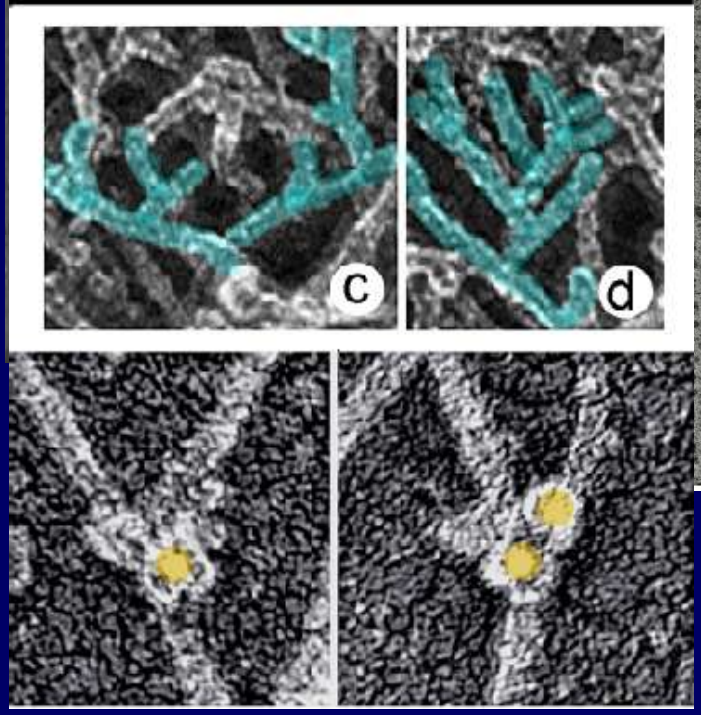
R. Robinson et al
(Science, 2001)

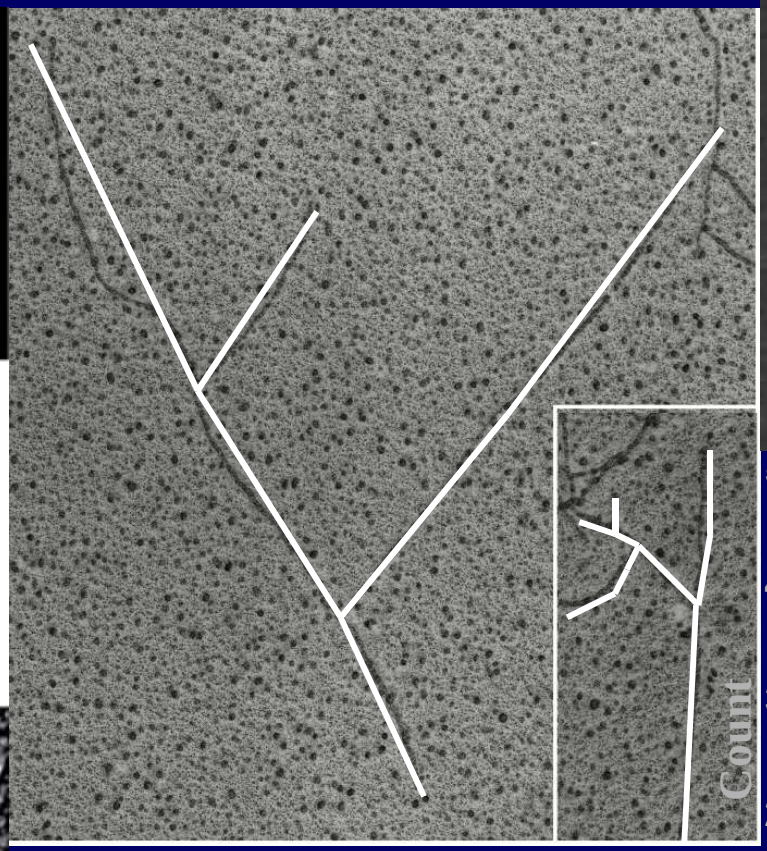


(Miki et al., 1998; Machesky and Insall, 1998; Rohatgi et al., 1999; Egile et al., 1999;
Kim, Rosen et al., 2000; Prehoda et al;, 2000)

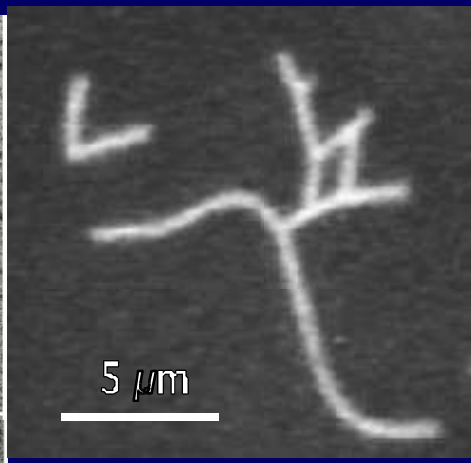
Filament branching array in lamellipodia

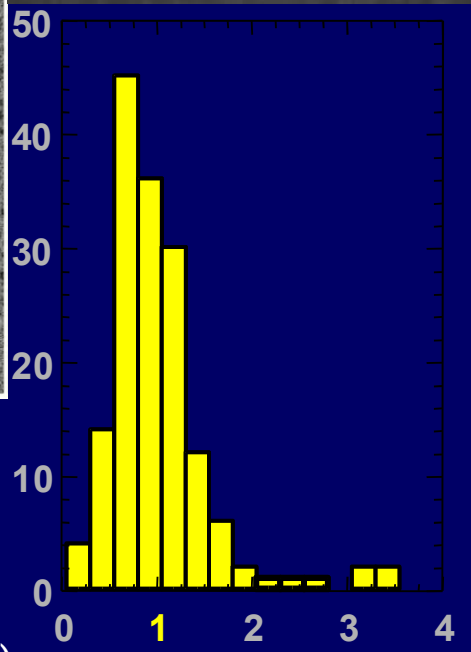






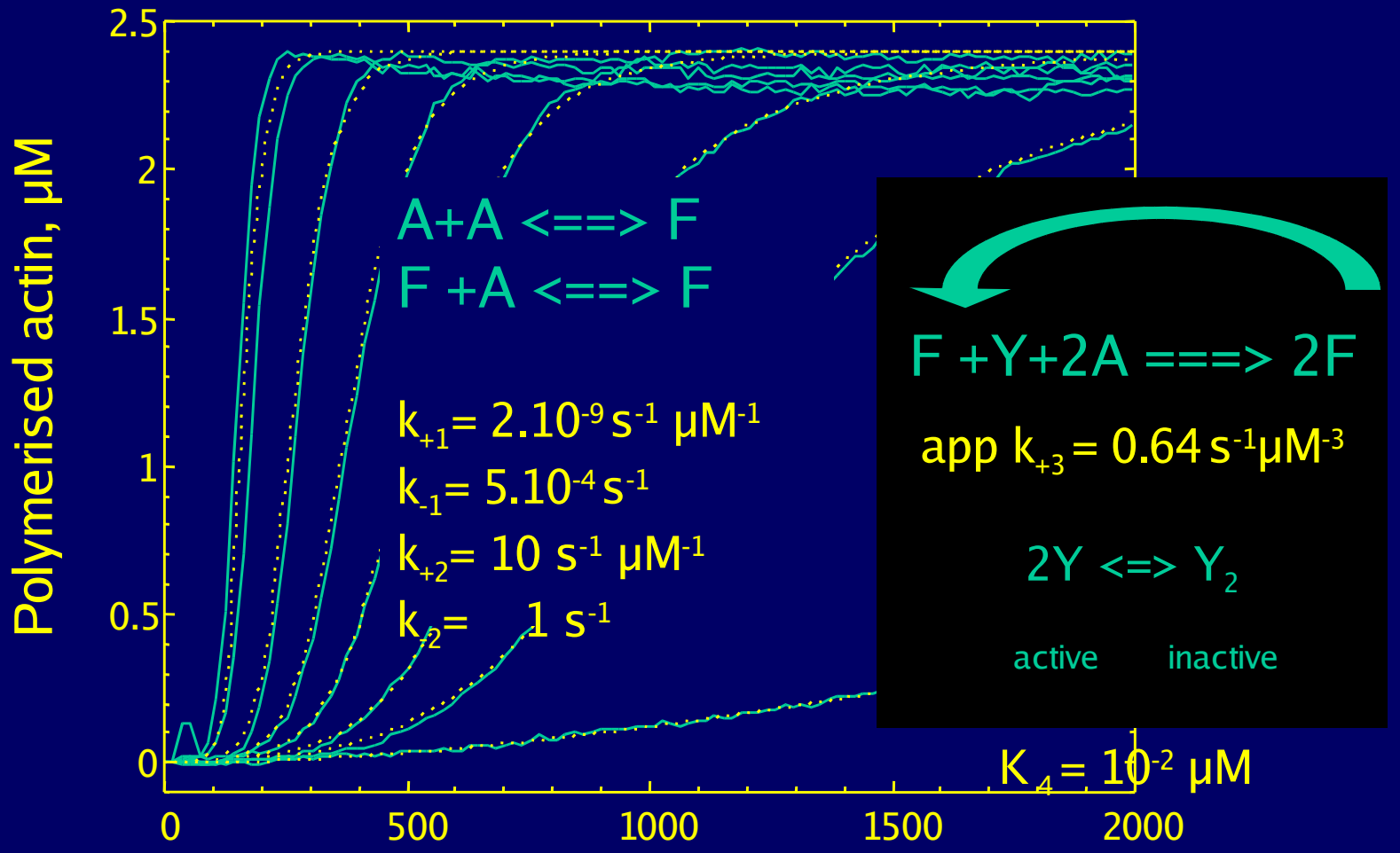
Branching





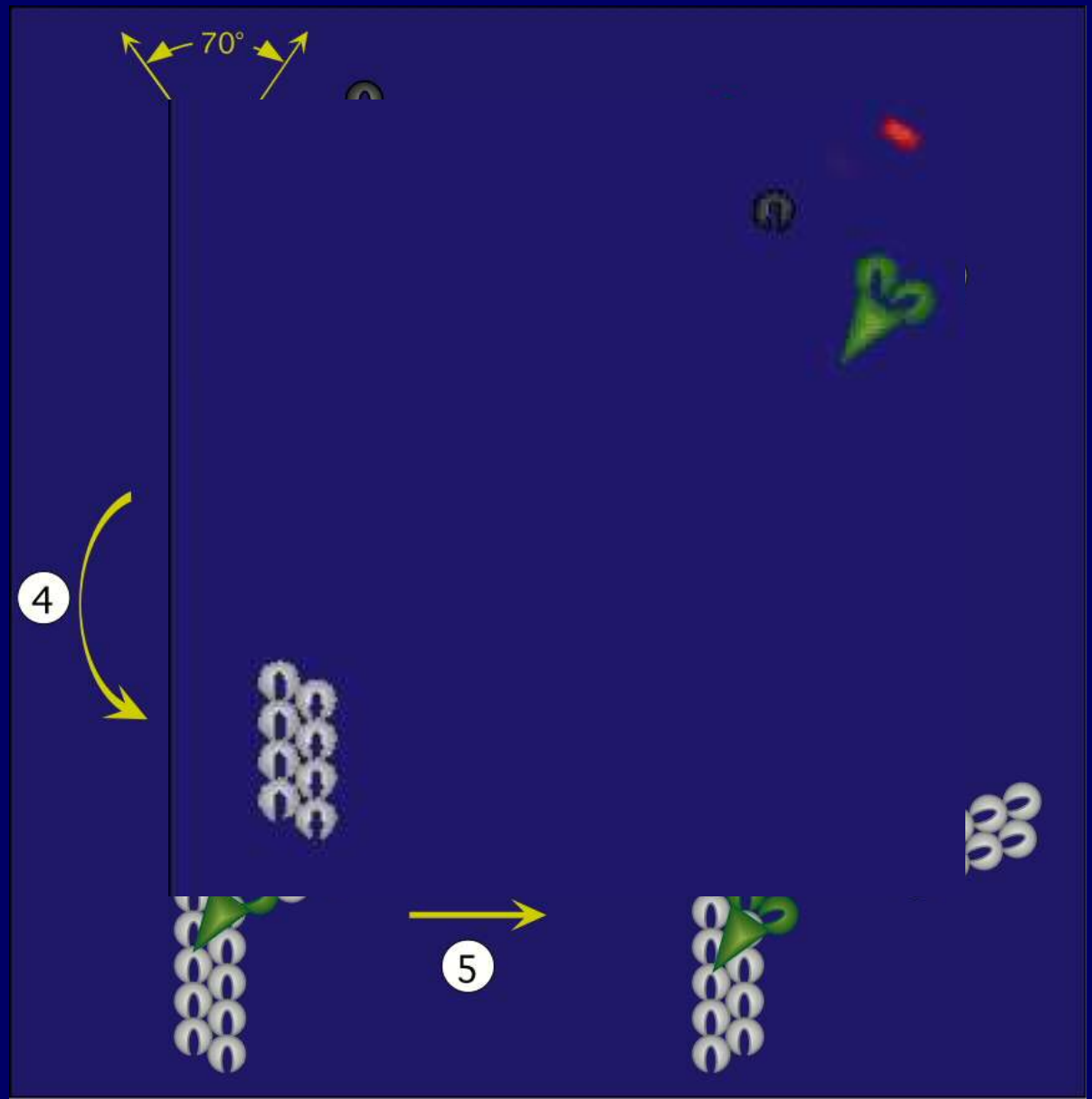
(T. Svitkina and G.G. Borisy, 1999; Blanchoin et al.; Pantaloni et al., 2000)

Modeling barbed end branching by Arp2/3 complex: un autocatalytic process

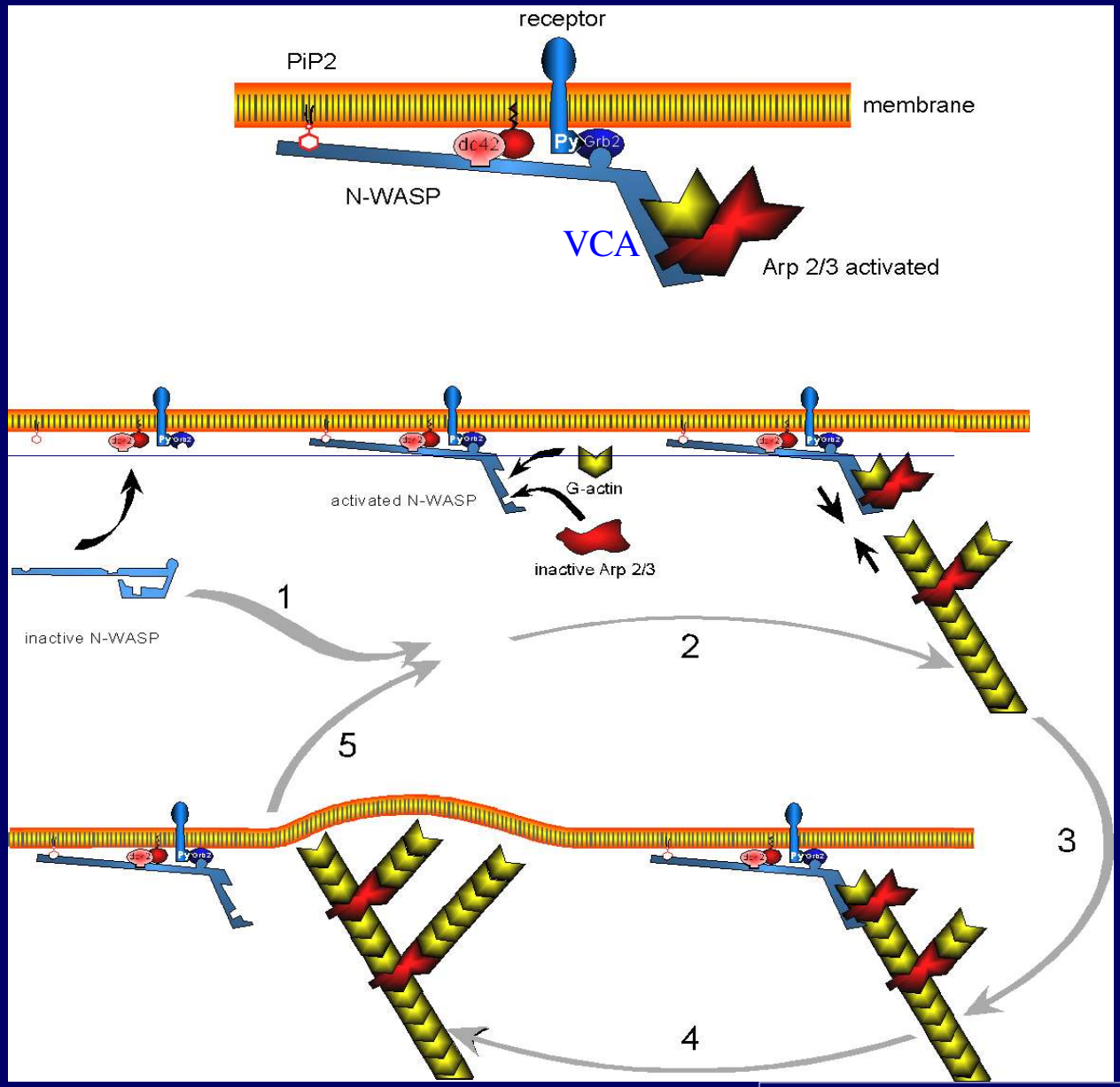


Arp2/3: Branching Mechanism

(Pantaloni et al., NCB 2000)



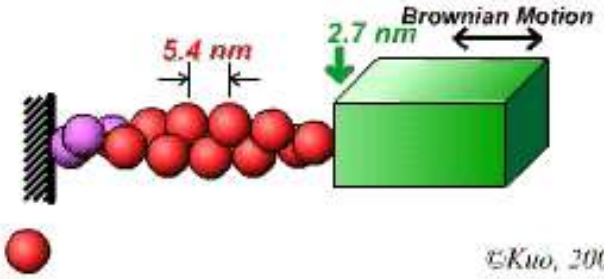
Cycles of filament attachment-detachment are coupled to branching

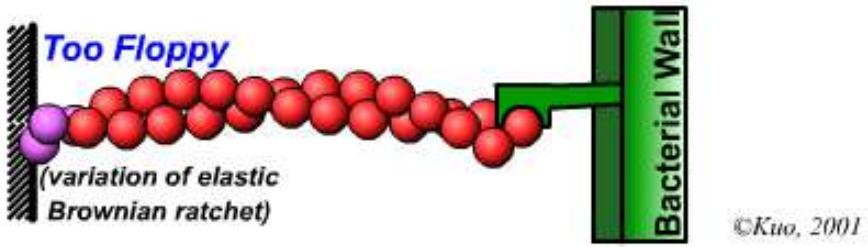


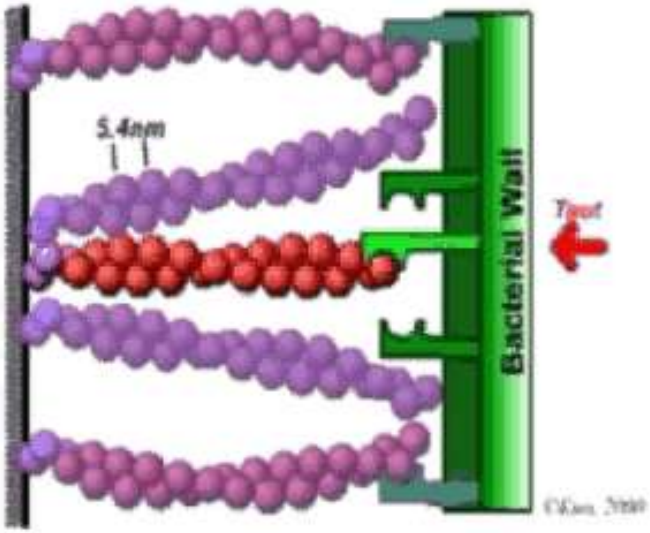
Pantaloni et al., NCB 2000; Science 2001

Actin polymerization and force production: Evolution of the Brownian Ratchet

(Oster and Mogilner, 1996-2003)







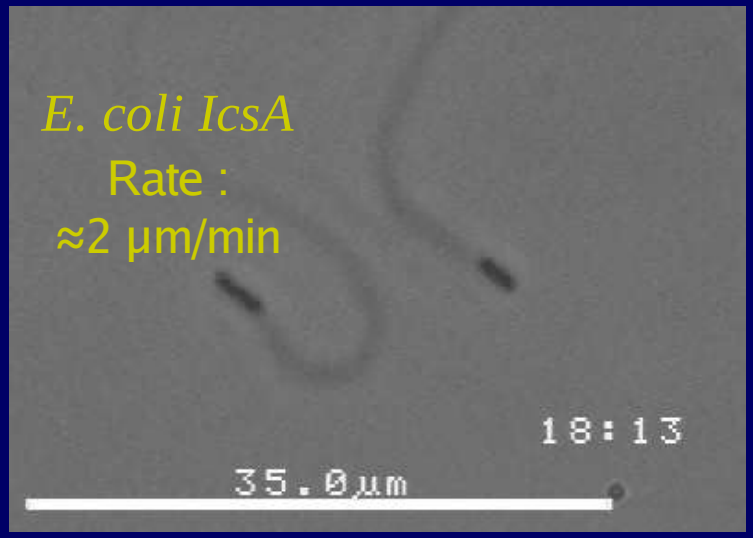
Reconstitution of actin-based movement from pure proteins (Loisel et al., Nature 1999)

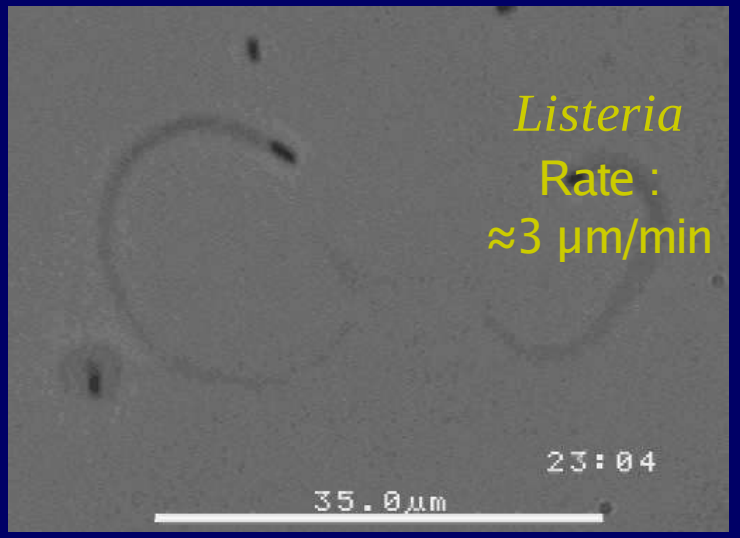
- Treadmilling of filaments feeds movement
- Functions required for movement:

3) Site-directed generation of barbed ends
   by N-WASP (resp. ActA)-activated Arp2/3
2) Chemostat maintaining a high steady-state concentration of ATP-G-actin : Actin, ADF/cofilin, profilin, Capping protein

- Movement results from a balance between t creation of new growing filaments (branching) and death of these filaments (capping).

Movement of *E. coli* (IcsA) and *Listeria monocytogenes* with pure components.





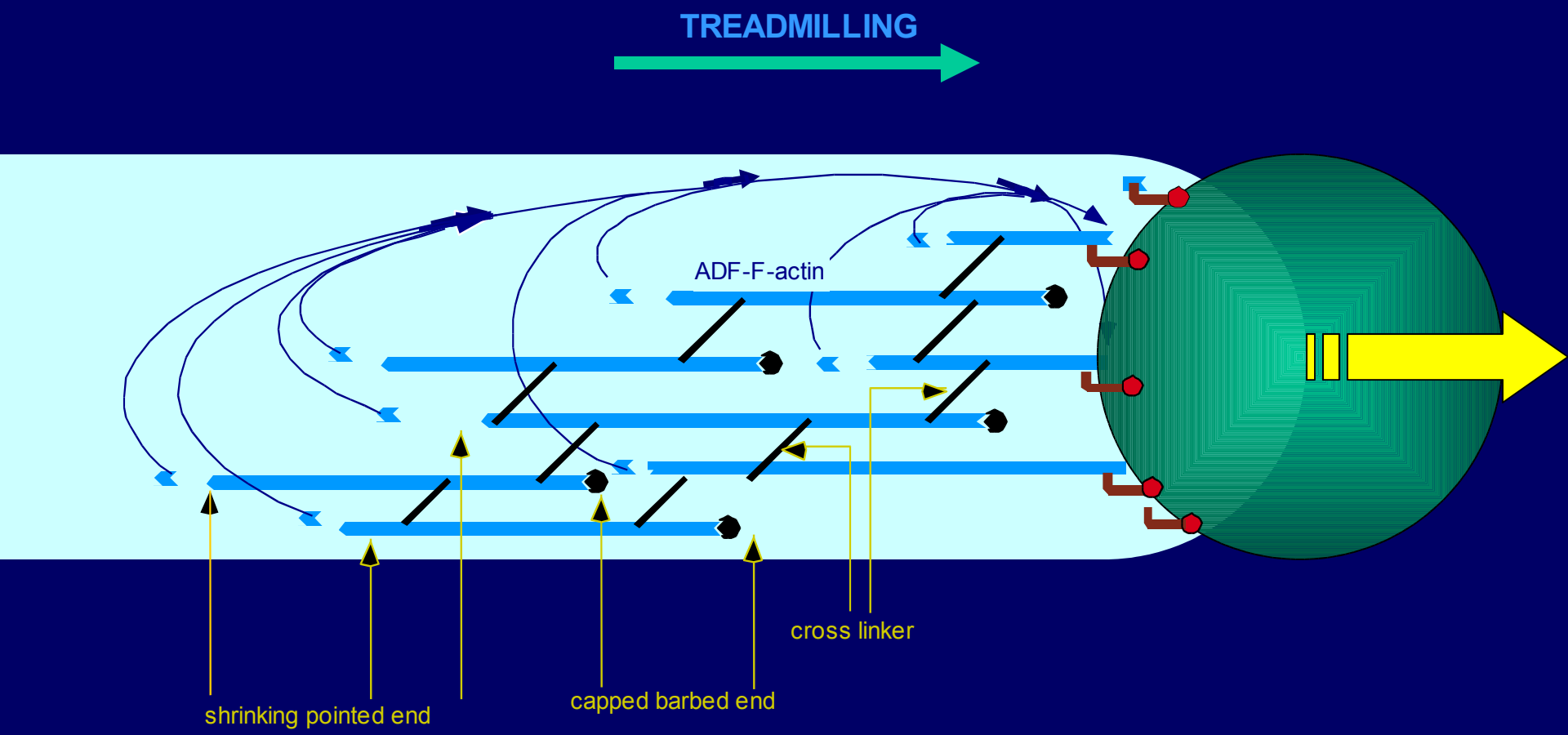
Essential Proteins :

	IcsA-bound
N-WASP	
Arp2/3	0.1 µM
Capping Protein	0.1 µM
ADF	2 µM
ATP-actin+F-actin	8 µM

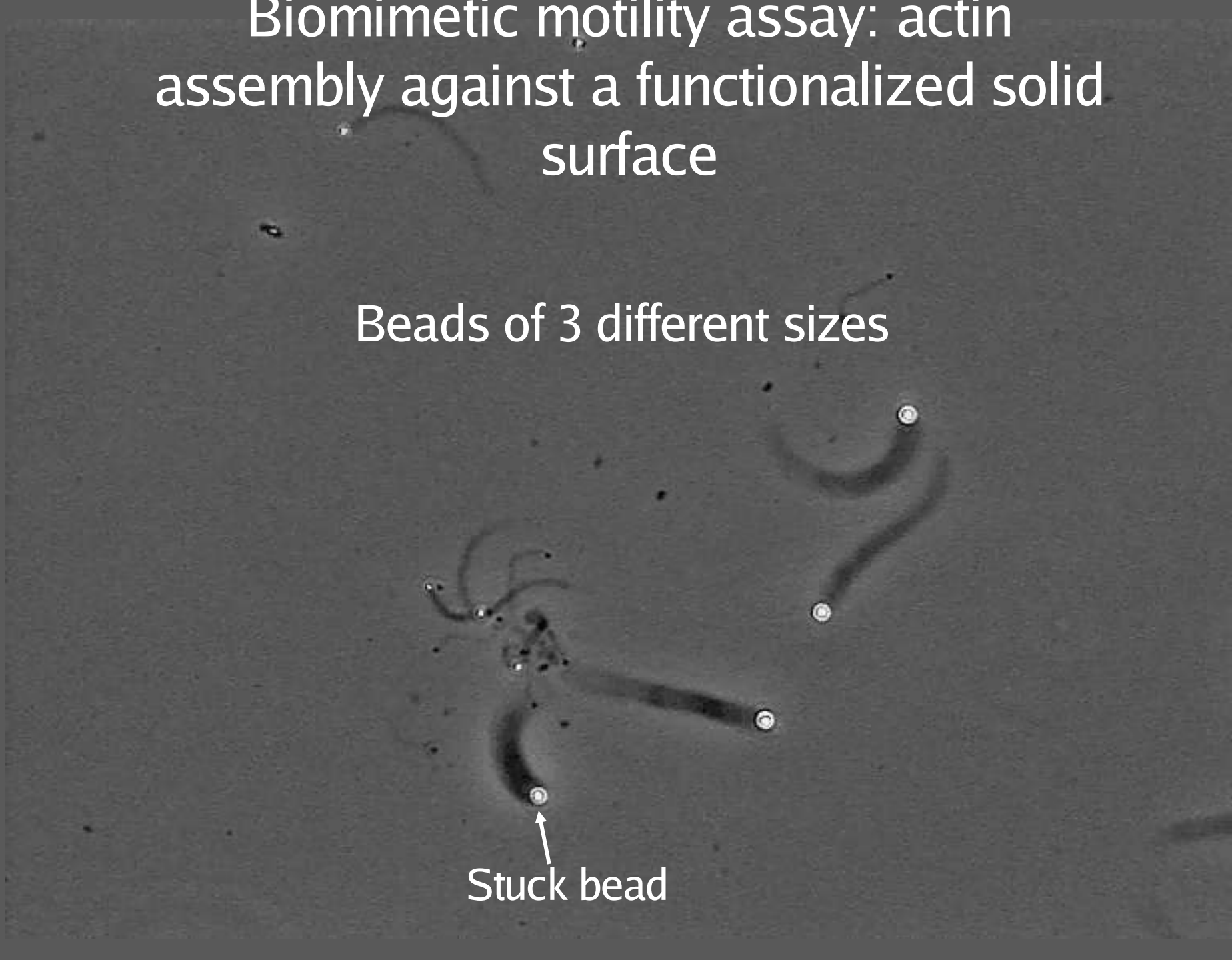
Useful Proteins :

Profilin	2 µM
α-actinin	0.5 µM
VASP	0.1 µM

Mimicking Lamellipodium Protrusion

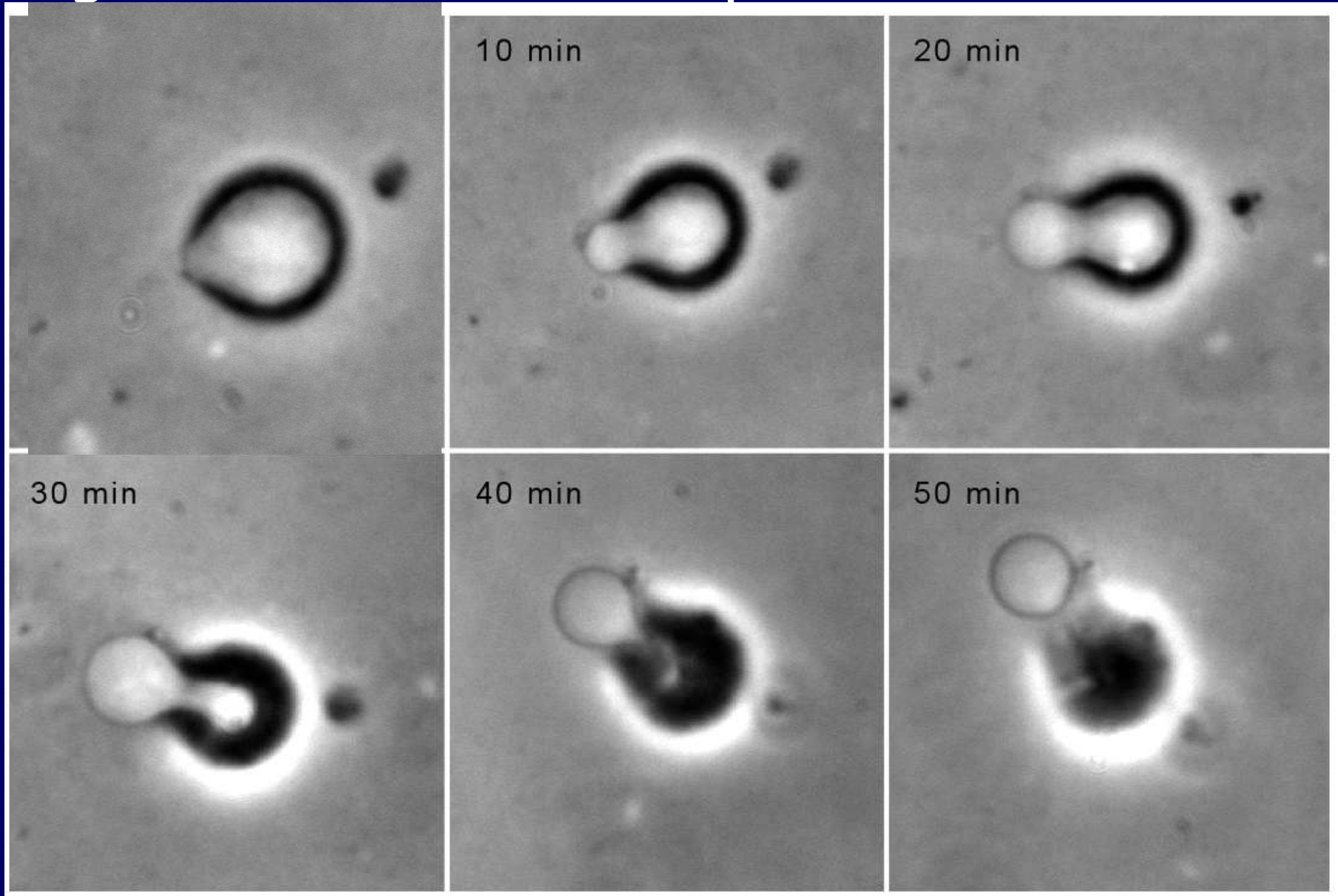


Biomimetic motility assay: actin assembly against a functionalized solid surface

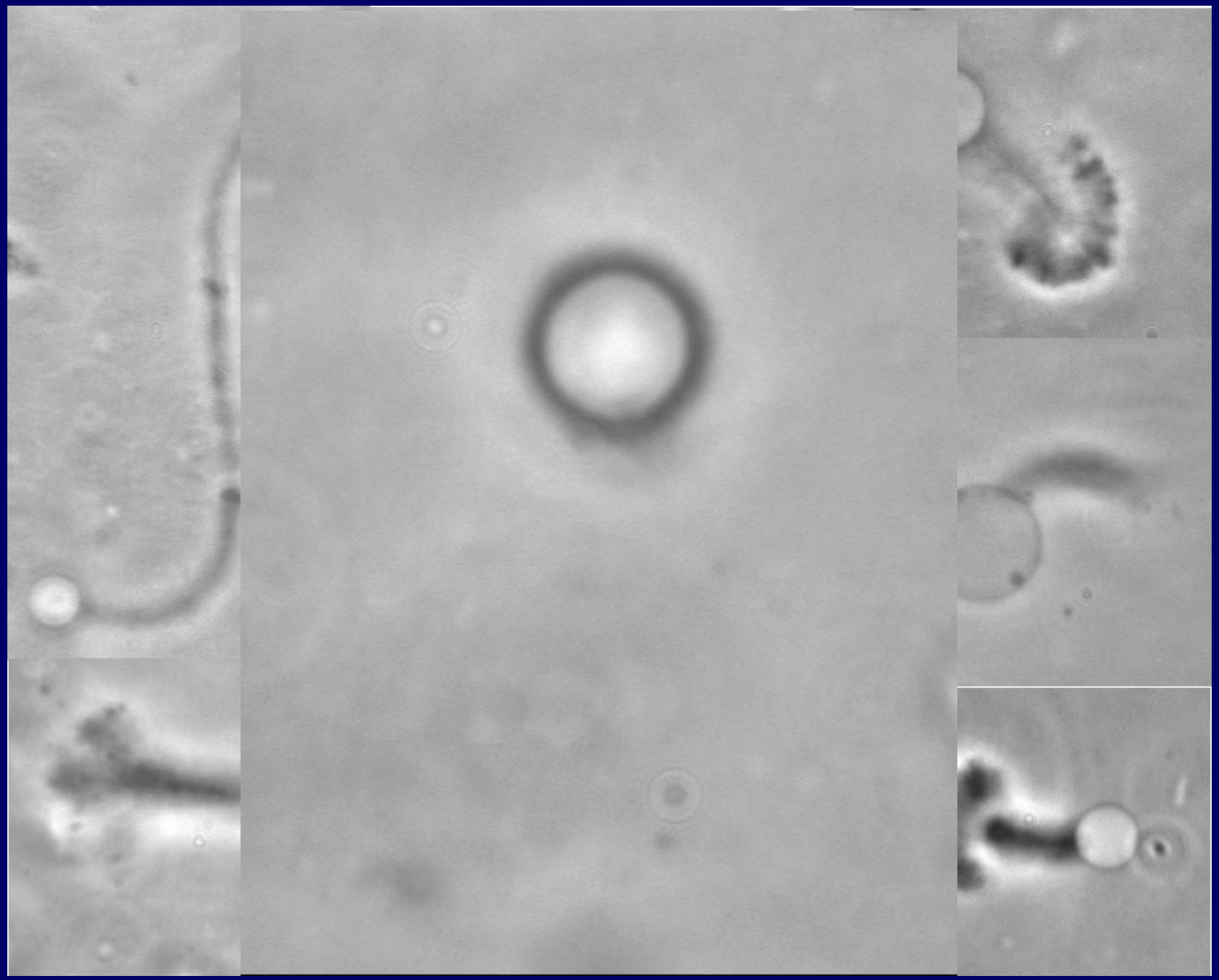


A break of symmetry in the actin meshwork leads to a polarized actin tail and movement

Biomimetic motility assay: actin assembly against a functionalized lipid membrane (GUV)



Actin tail forms and propels the liposome following break of symmetry

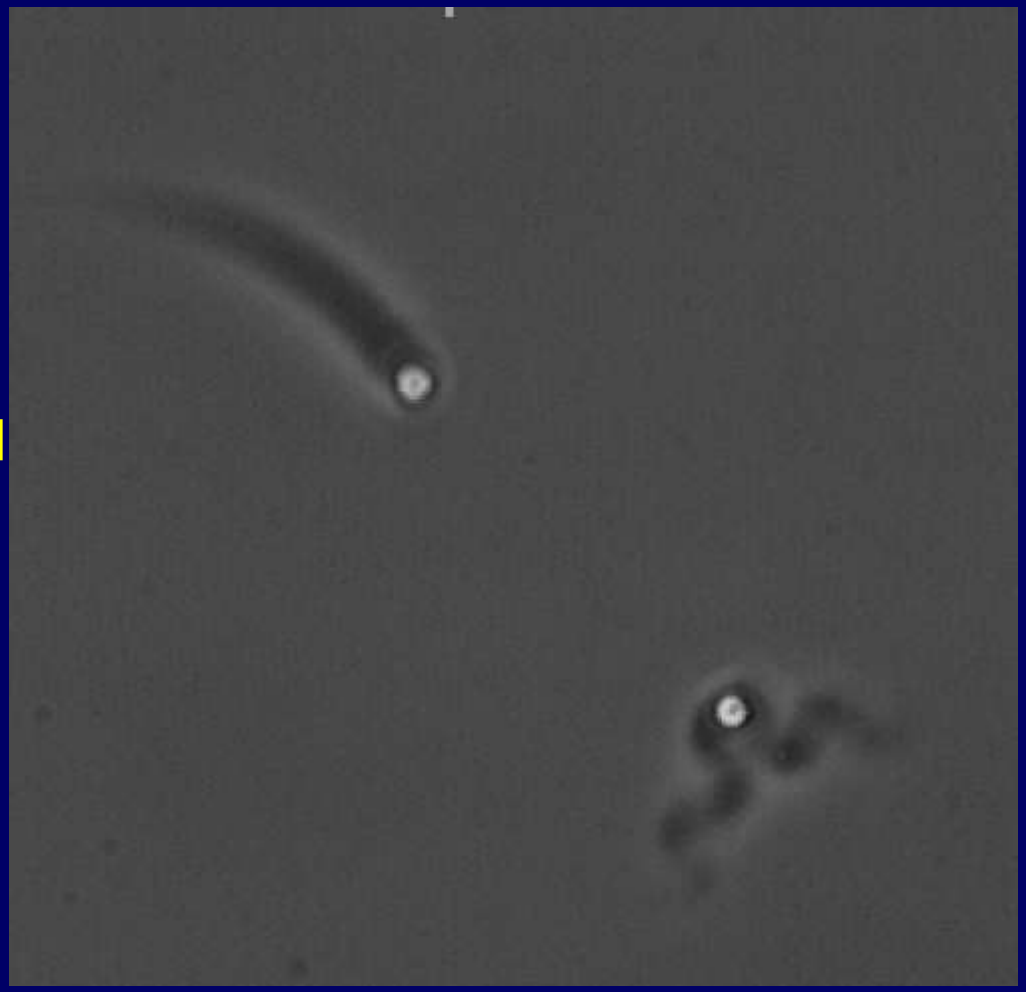


03:18

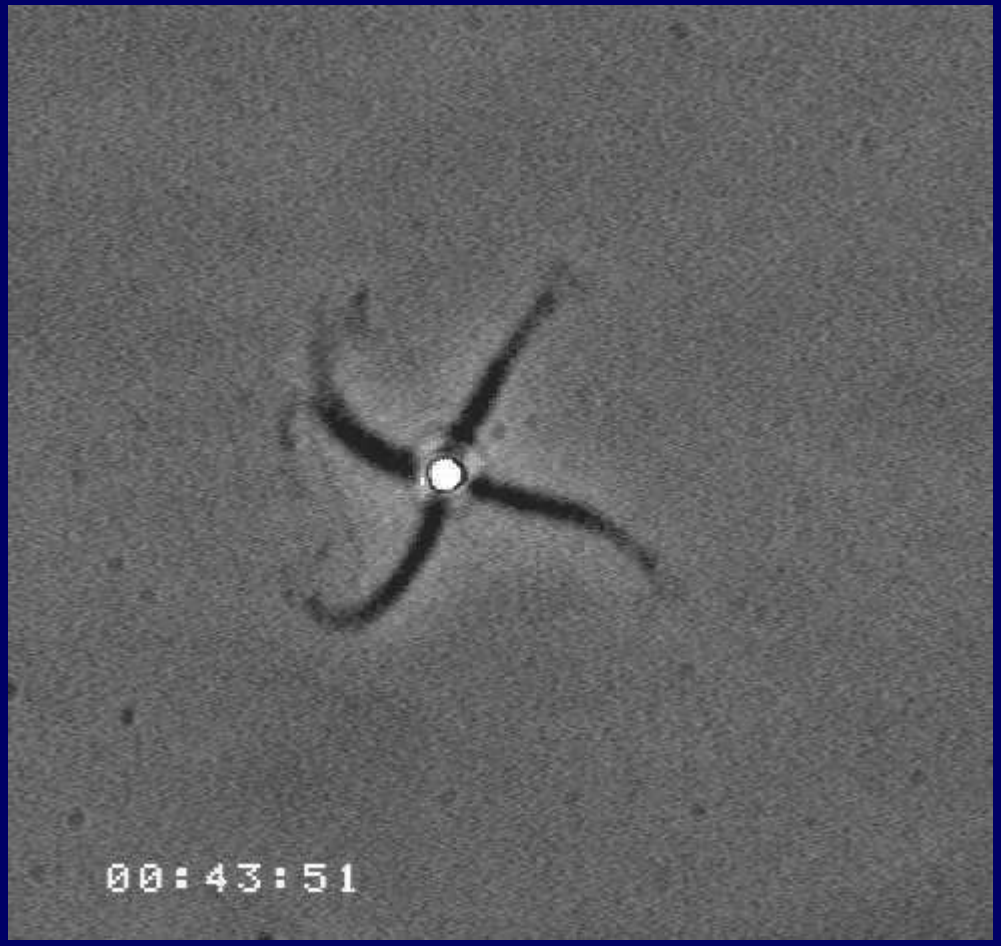
Encounters of the third kind

Motility medium :

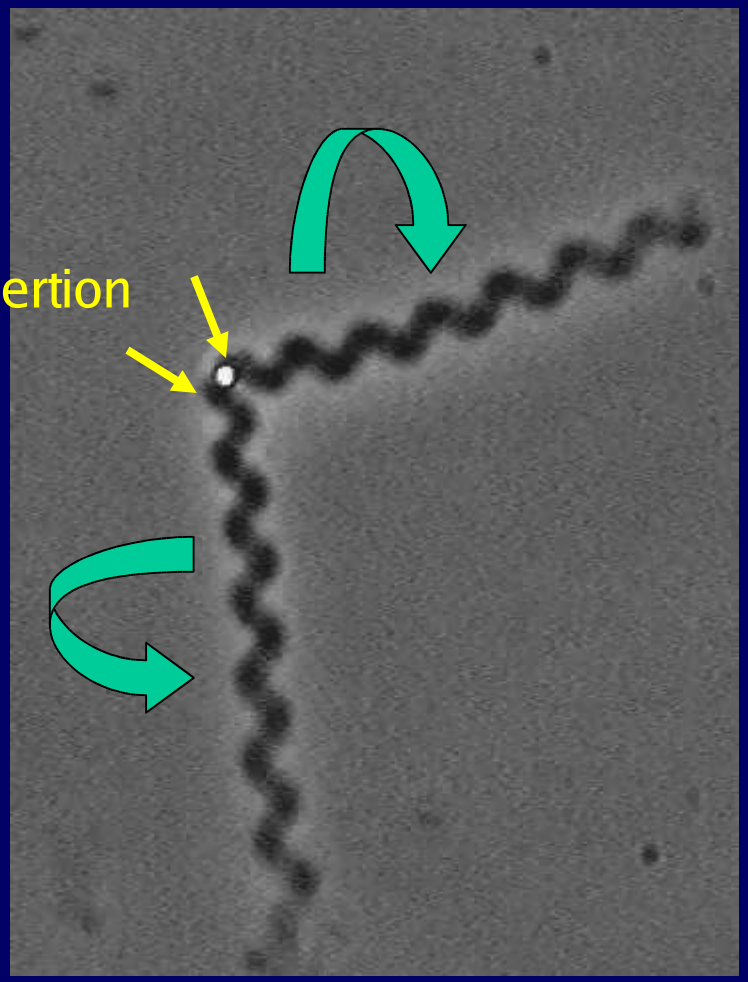
N-WASP	bead-bound
Arp2/3	0.1 μM
Capping Protein	0.1 μM
ADF	2 μM
ATP-actin+F-actin	8 μM
Profilin	2 μM

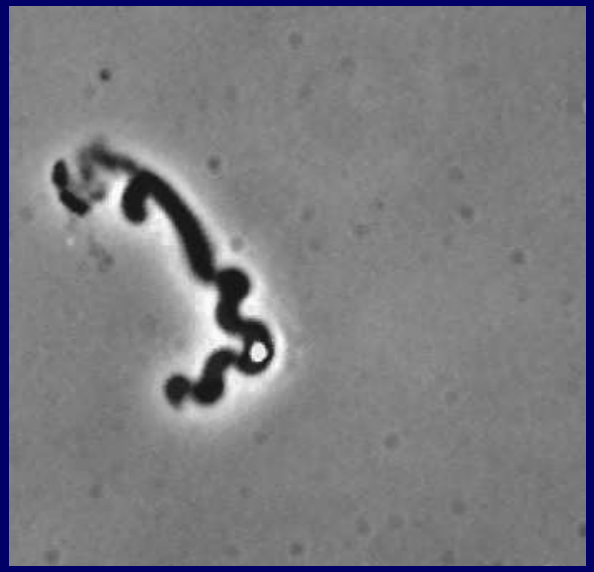


Four Symmetric Comets

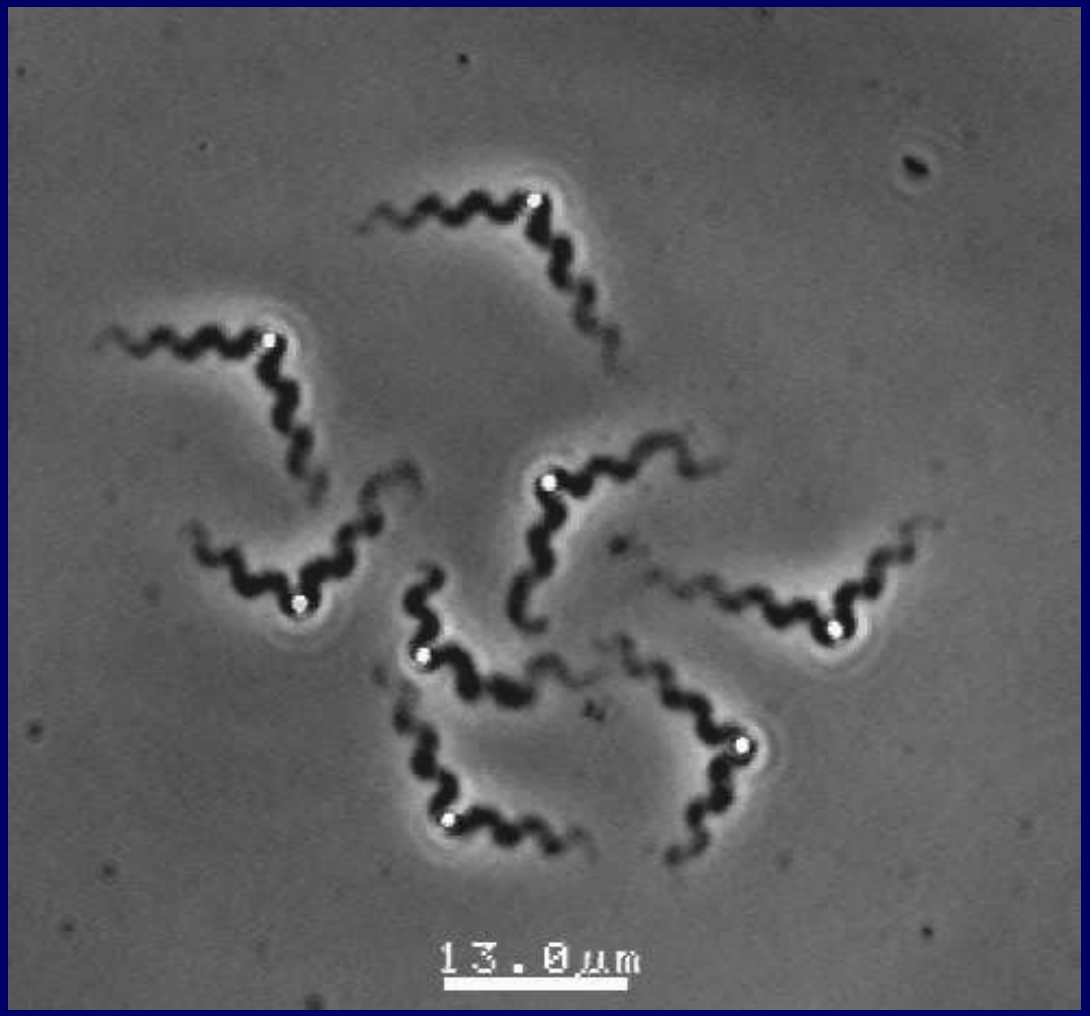


The two helices rotate in register and display opposite handedness

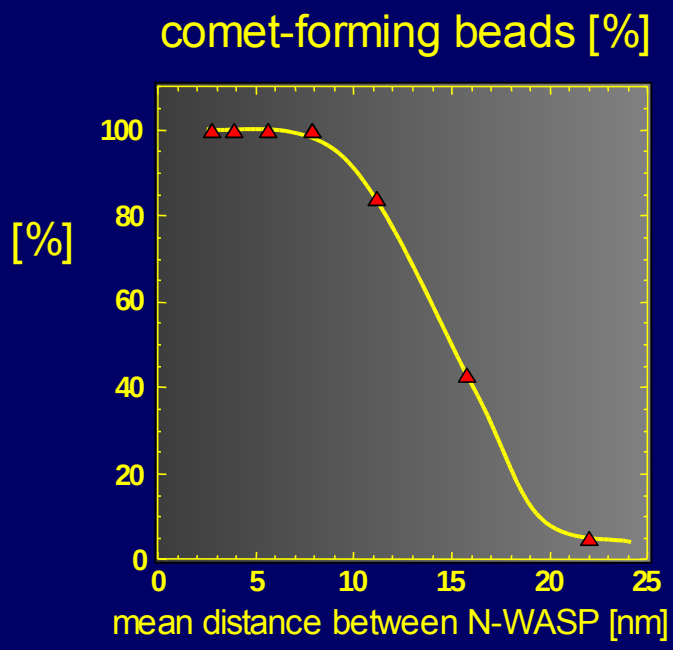




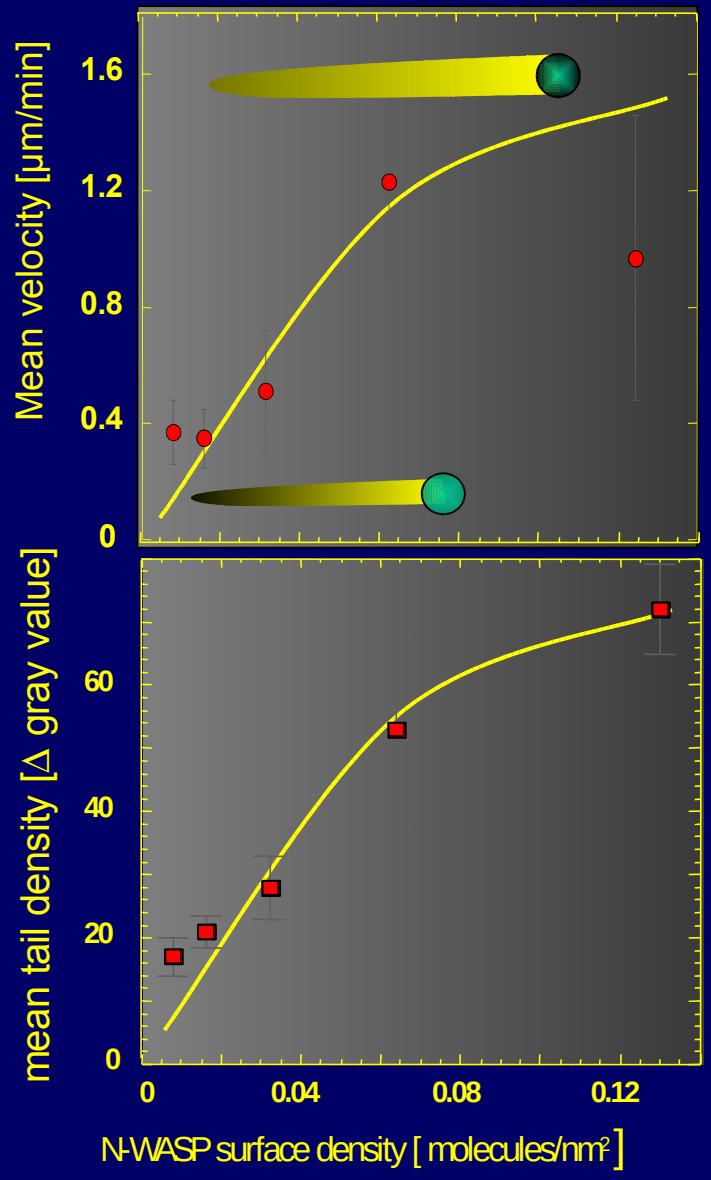
The helical parameters of the actin tails depend on the geometry of the microsphere



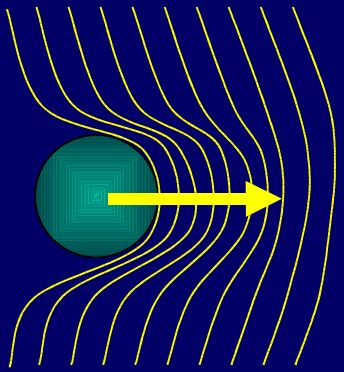
The surface density of N-WASP affects bead motility

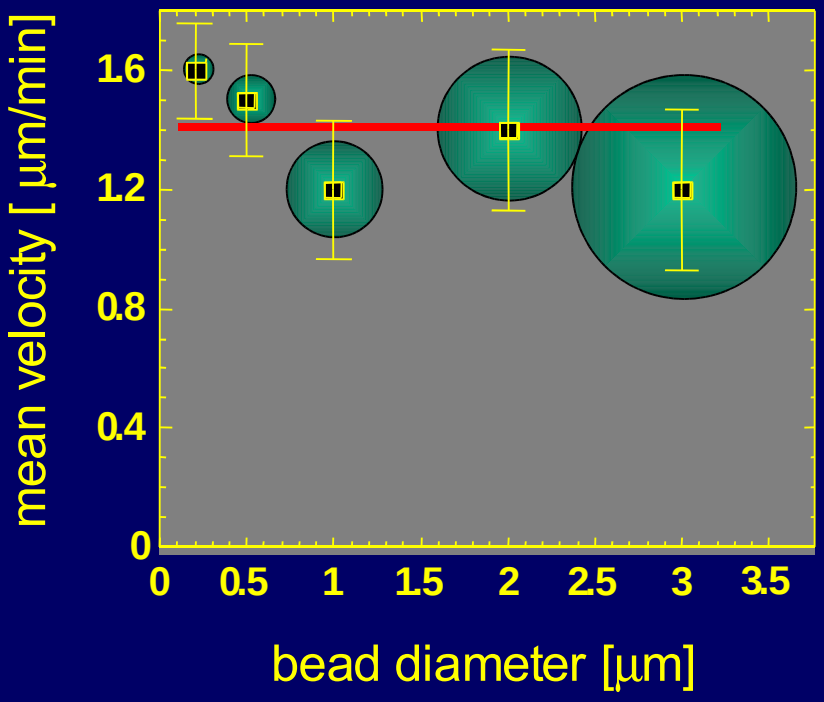


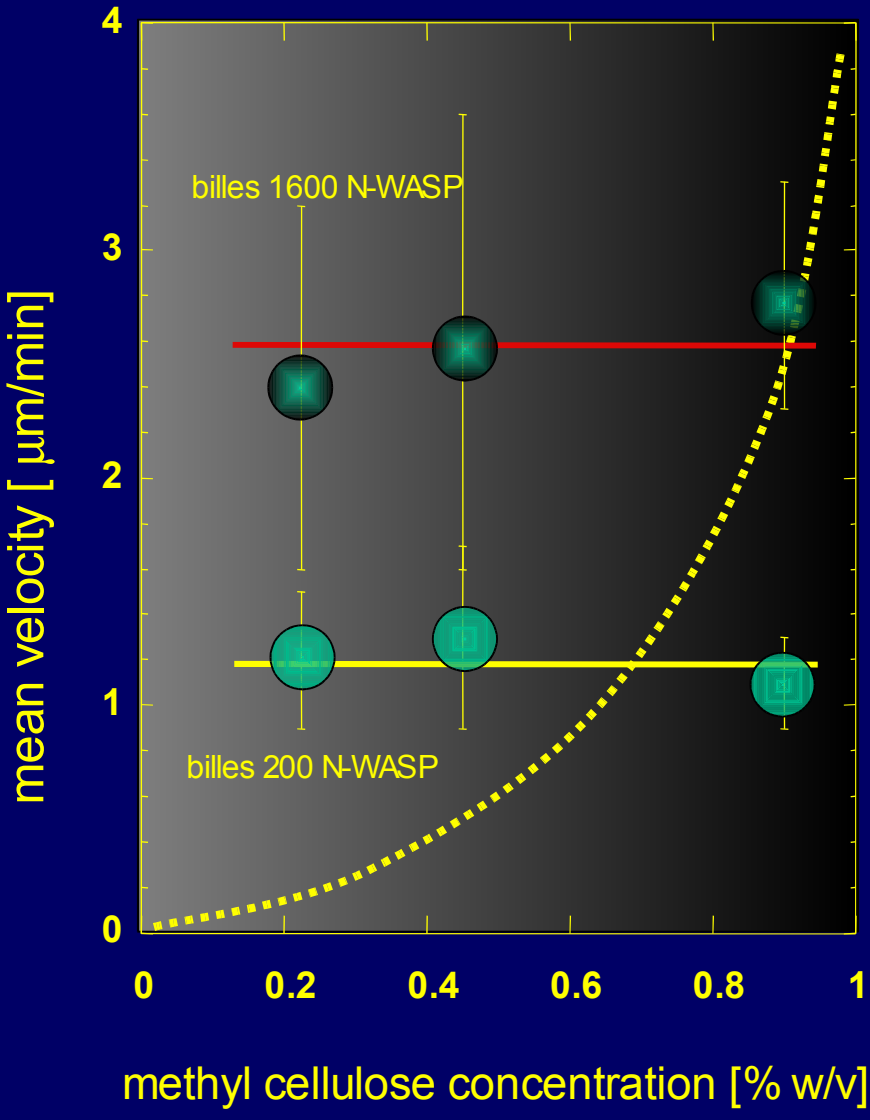
- Movement requires a threshold of N-WASP density
- Velocity is proportional to filament number, i.e. to N-WASP density



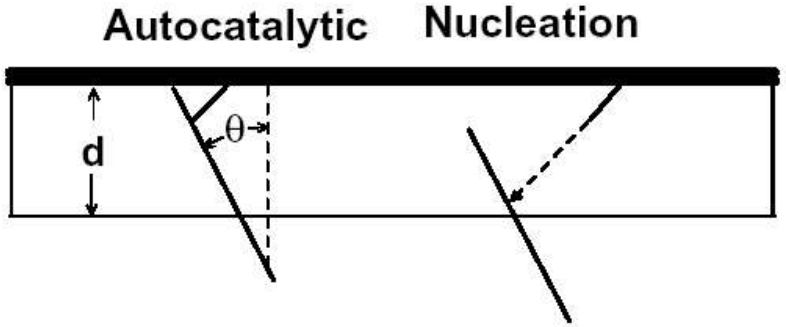
Effect of external force on motility

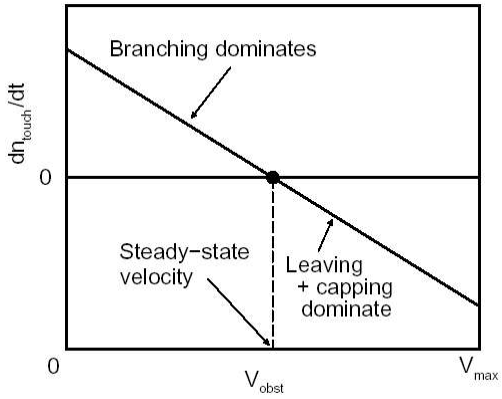






Simulation of actin-based motility: balance between filament branching and capping (A.E. Carlsson, 2003, Biophys. J.)





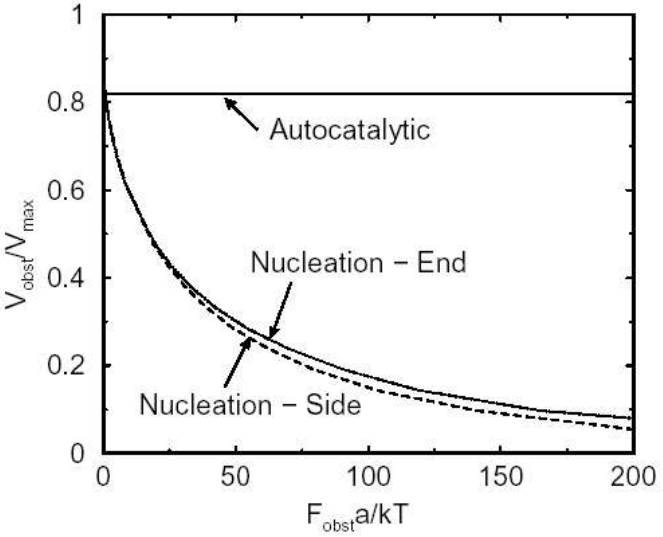
$$\frac{\partial n(\theta,t)}{\partial t} = k_{\rm br}\int_0^{\theta_{\rm max}} D(\theta,\theta')\nu(\theta')n(\theta',t)d\theta' - k_{\rm cap}n(\theta,t)$$
$$-H[V_{\rm obst}-v(\theta)][(V_{\rm obst}-v(\theta))/d+(\nu(\theta)k_{\rm br}-k_{\rm cap})]n(\theta,t),$$

$$dn_{\rm touch}/dt \geq (k^*_{\rm br}-k_{\rm cap})n_{\rm touch}(t),$$

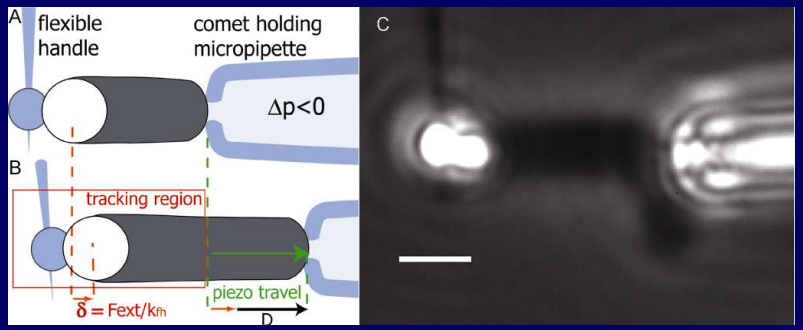
Autocatalytic branching :

1. Growth velocity is independent of applied force

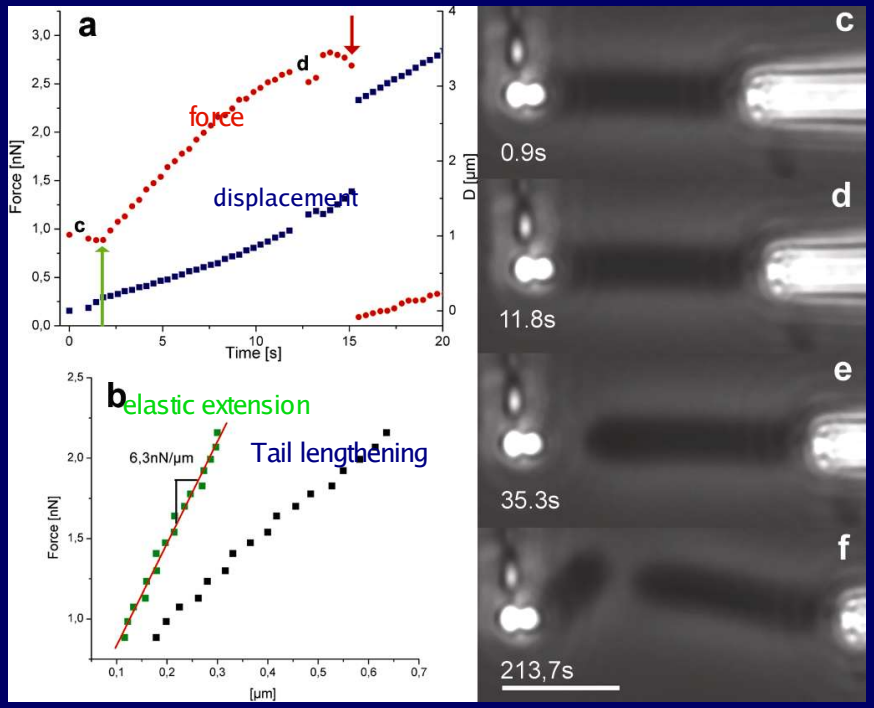
2. Branch spacing decreases with capping



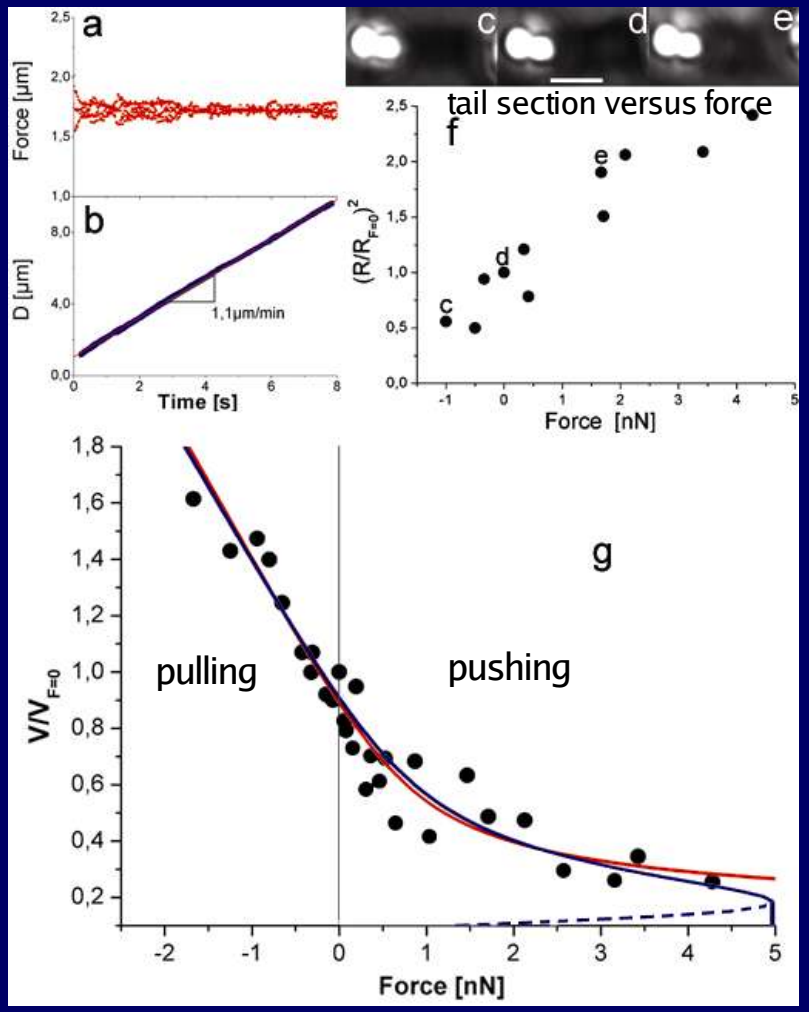
Measurement of force velocity relationship for actin-based propulsion



Experimental design



Fast pulling, detachment and regeneration of the actin tail

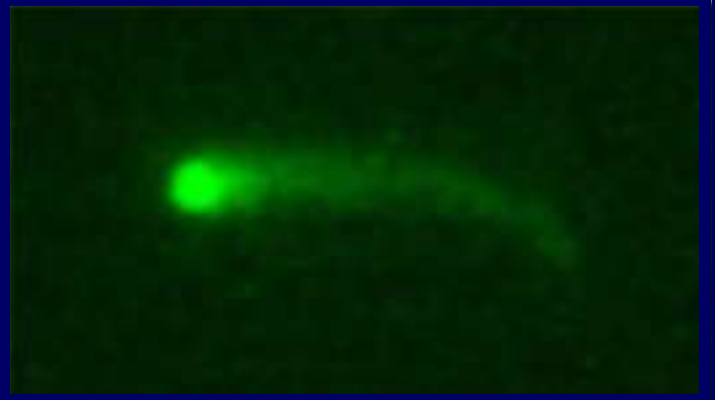


Force velocity diagram

Marcy et al., PNAS, 2004

Arp2/3 incorporates in actin tails upon barbed end branching at the surface of N-WASP coated beads

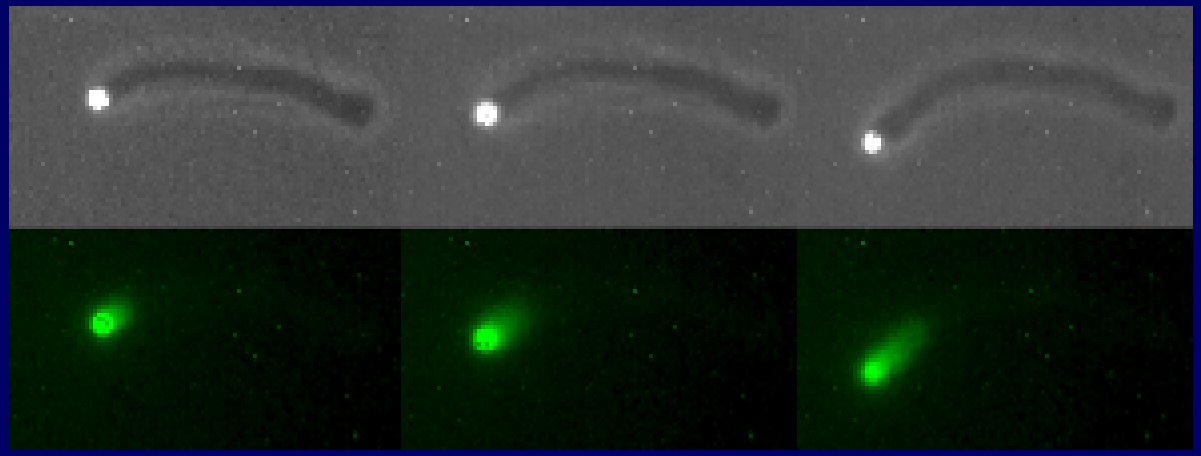
Rhodamine-actin



Alexa green-Arp2/3

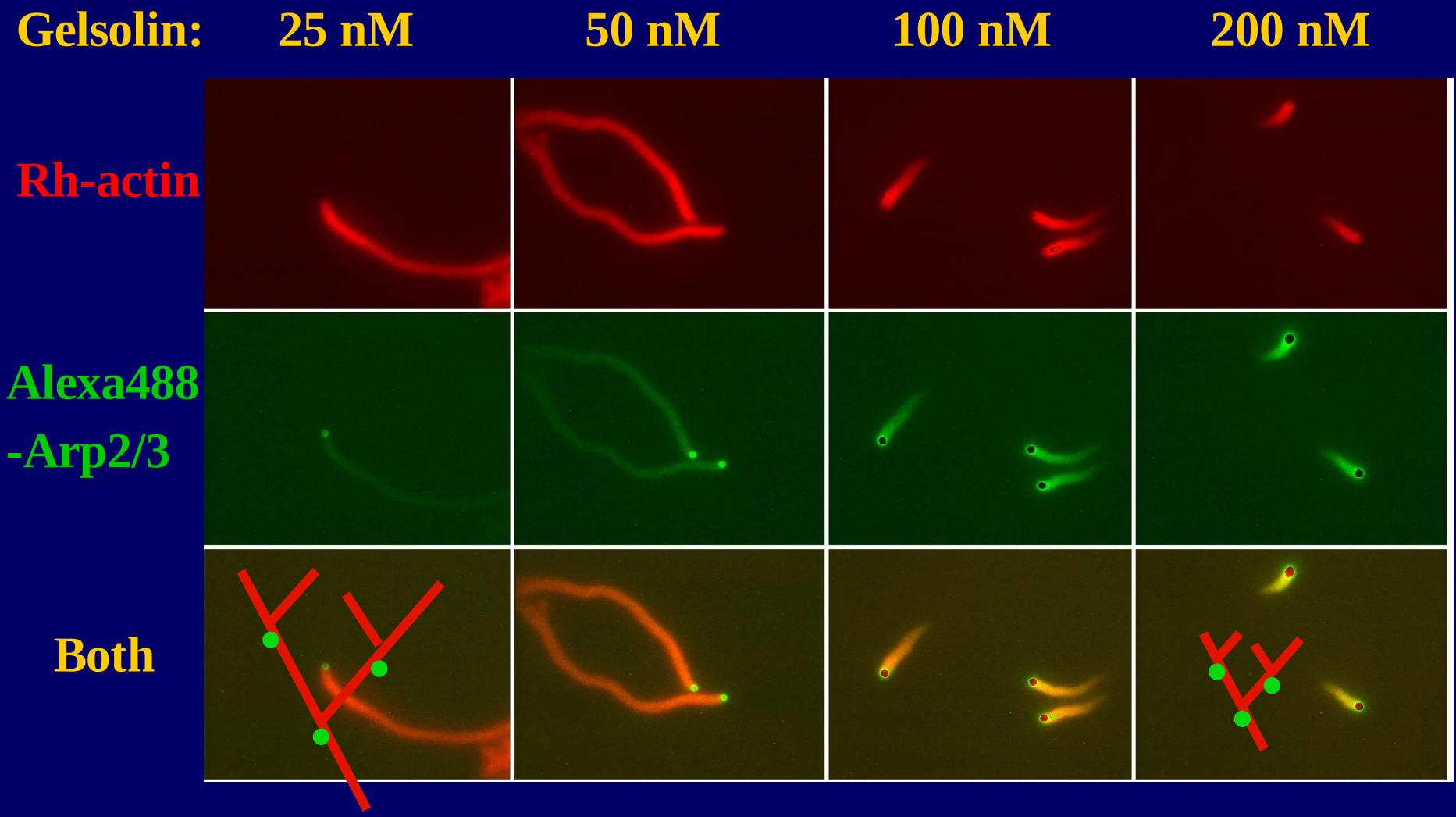
Phase contrast

Alexa green-Arp2/3



Addition of Alexa green-Arp2/3

Branch spacing decreases steeply upon increasing capping *(Wiesner et al., JCB, 2003)*

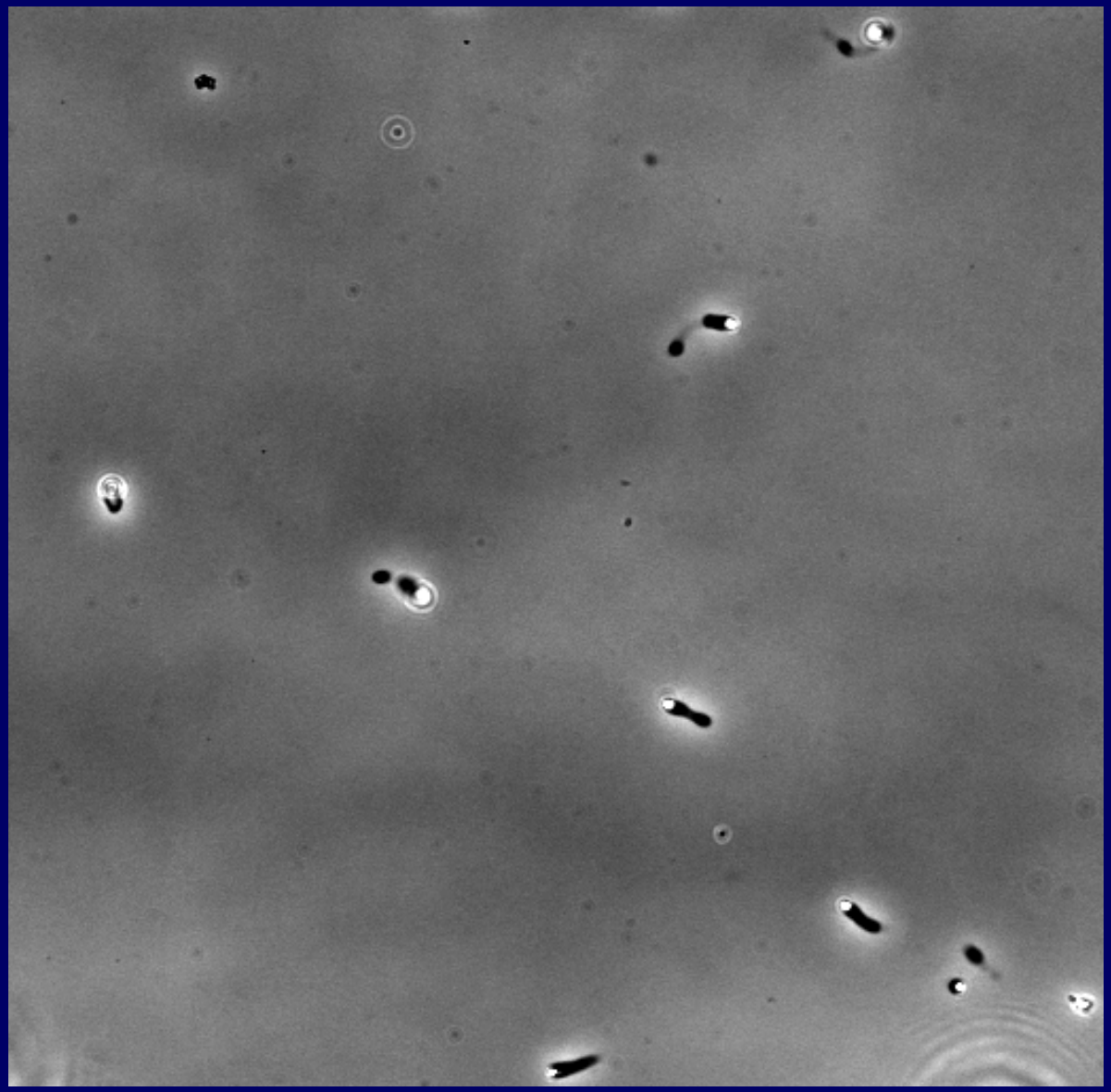


Conclusions

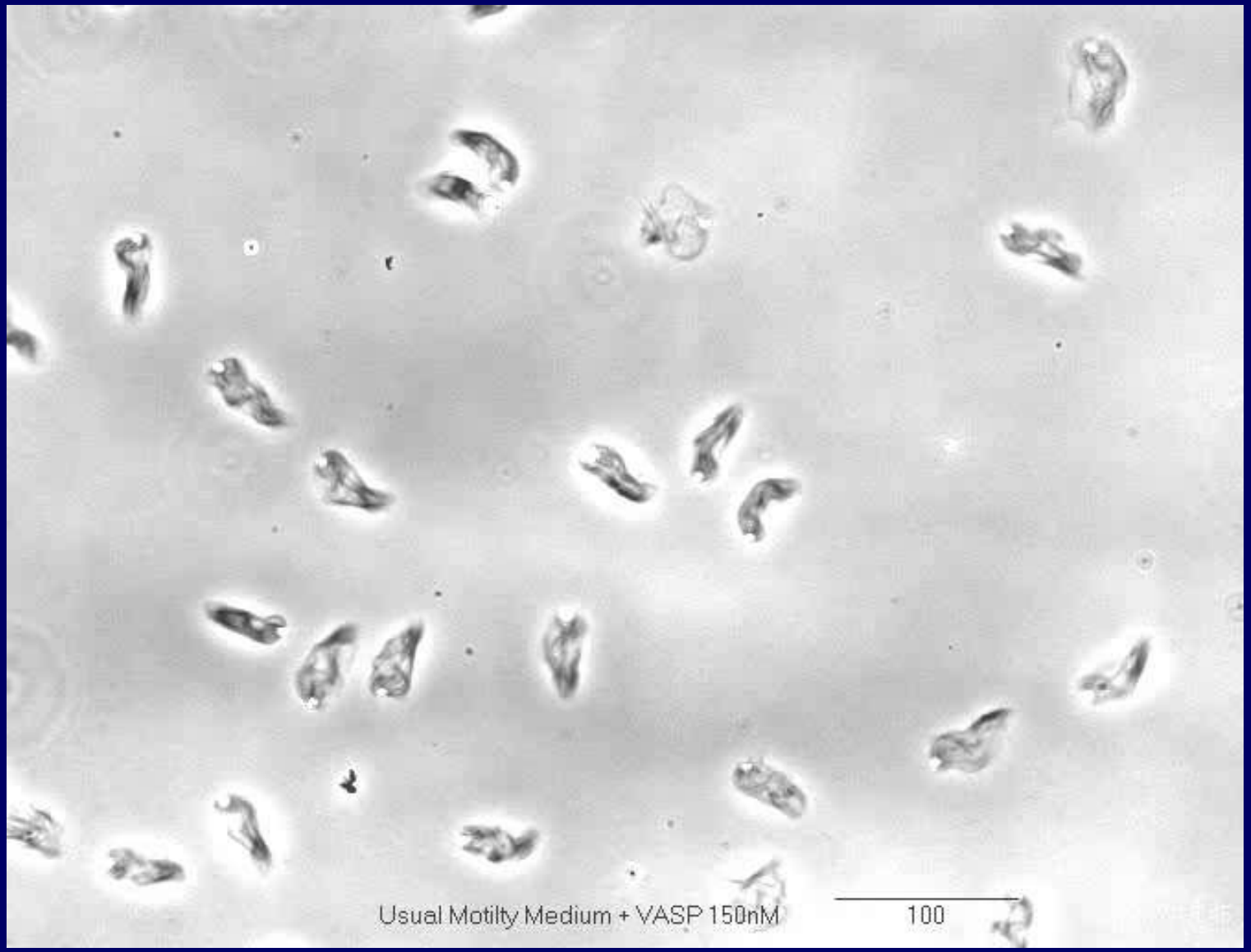
- The velocity of beads depends on the number of filaments pushing the bead.
- Movement is controlled by a balance between filament branching and capping (Carlsson's model).
- Velocity is not sensitive to external load (viscous drag), i.e. the force due to polymerization at the bead surface is balanced by the internal brake (friction) due to attached filaments.

Importance of the detachment of filaments following formation of the branched junction: role of VASP

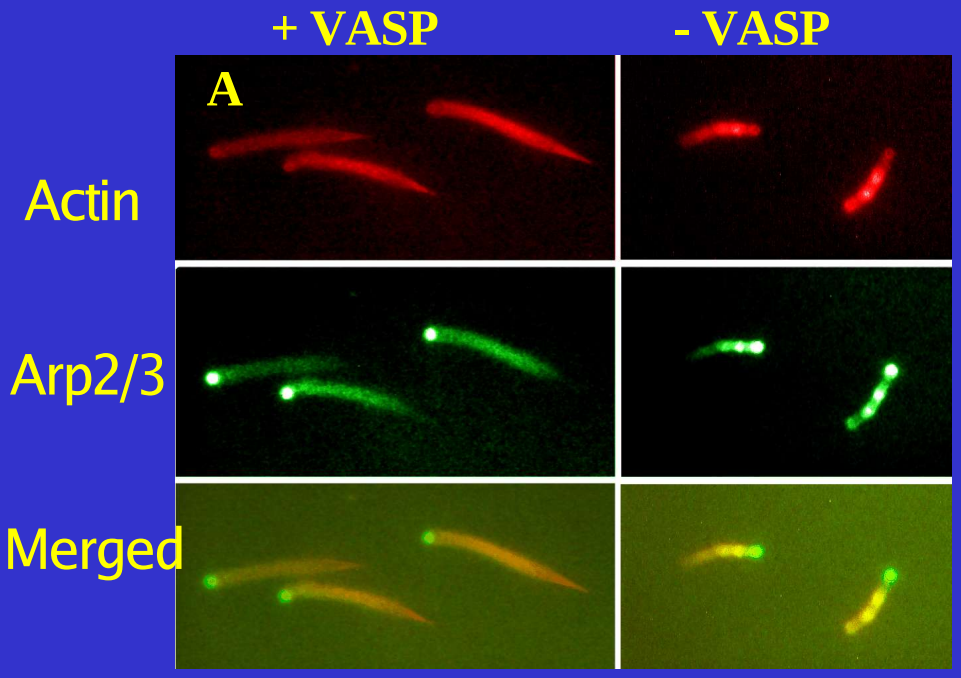
Effect of VASP on the motility of ActA-coated beads

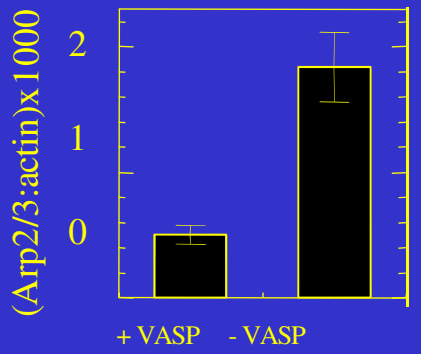


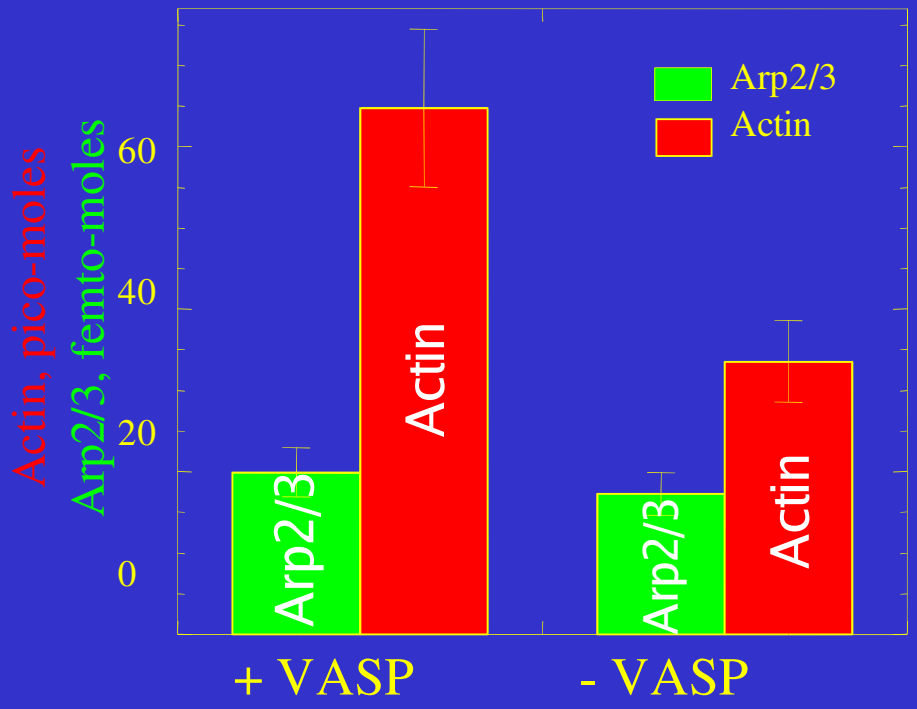
Effect of VASP on the motility of ActA-coated beads



VASP decreases the density of filament branching

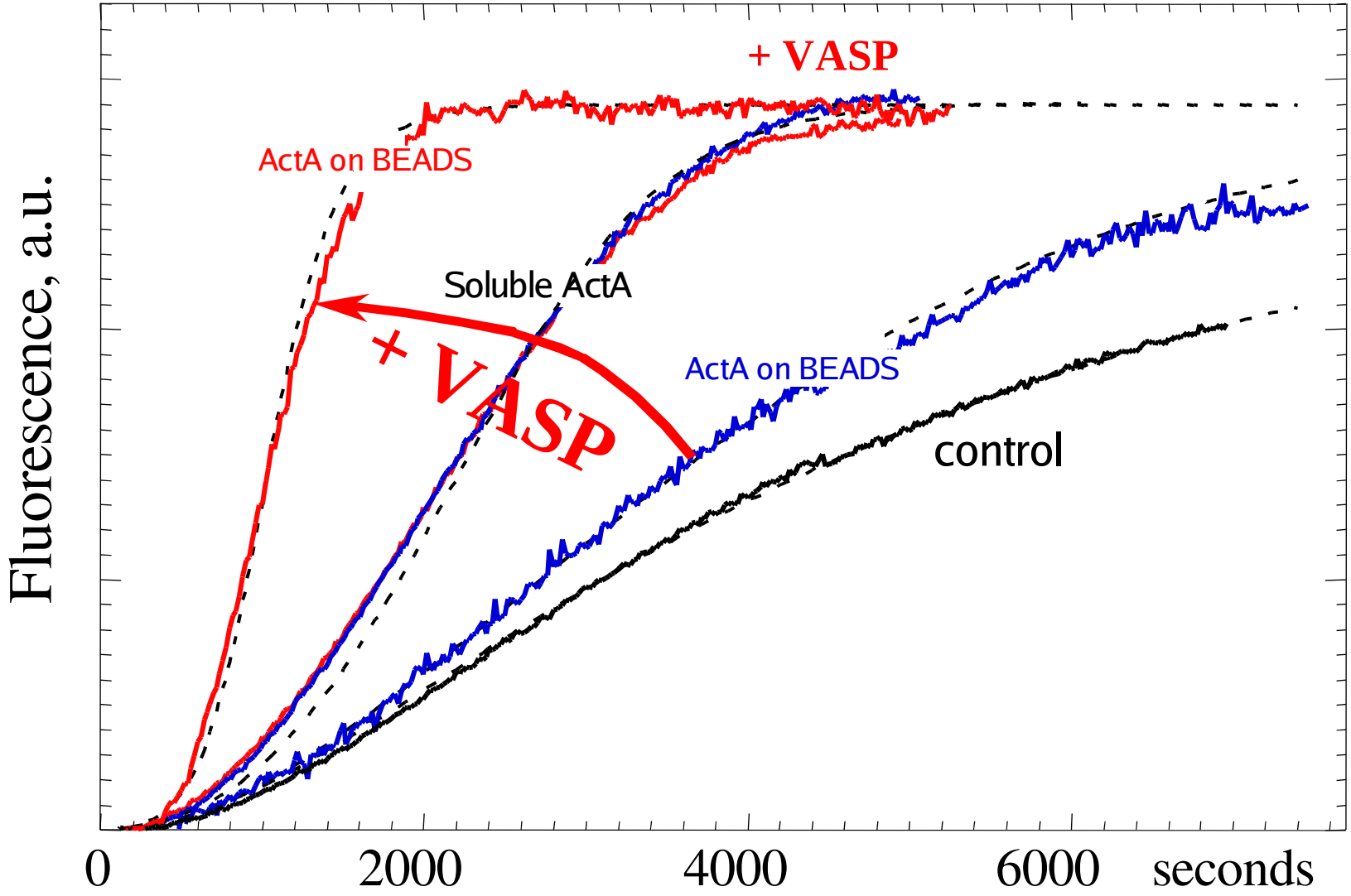




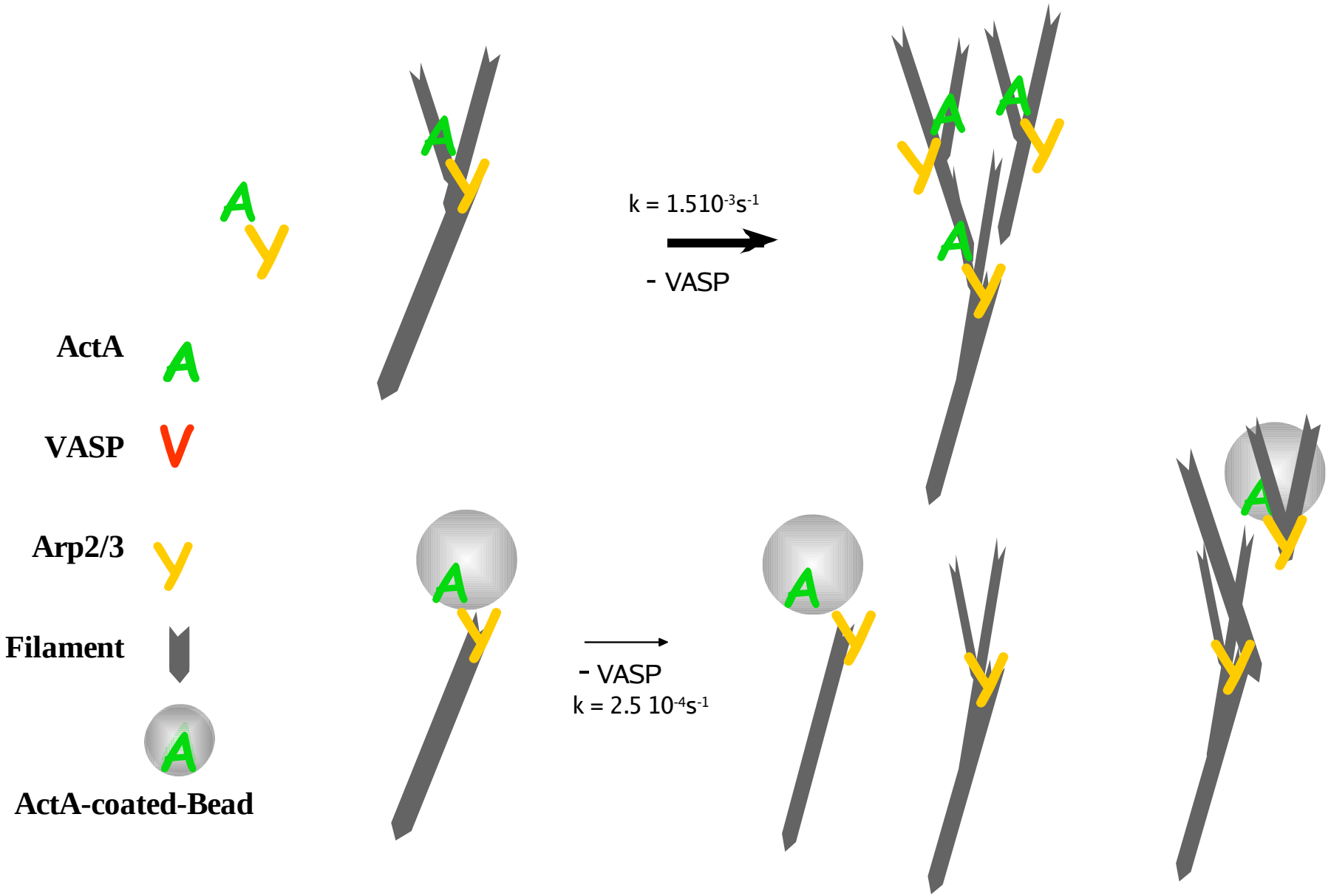


(Samarin, Romero et al., J. Cell Biol. 2003)

VASP enhances actin-based motility by accelerating filament detachment allowing growth after branching



Without VASP the rate of detachment of the branched junction from ActA, is slow.

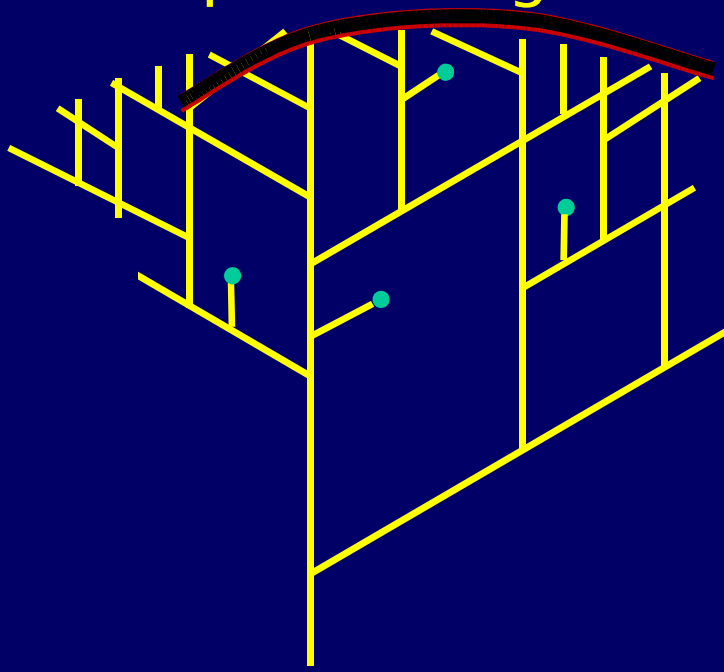


Actin-based motility

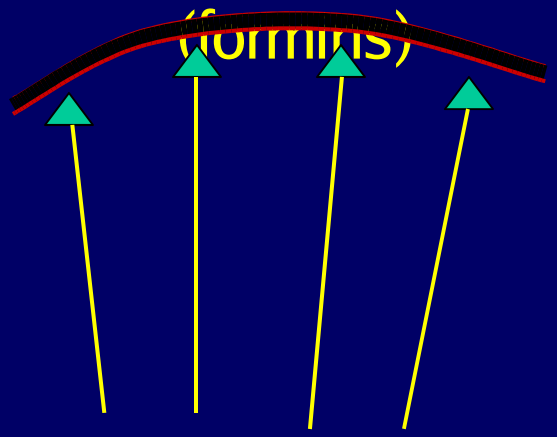
- Control of filament turnover
- Site-directed generation of new filaments:

2 mechanisms:

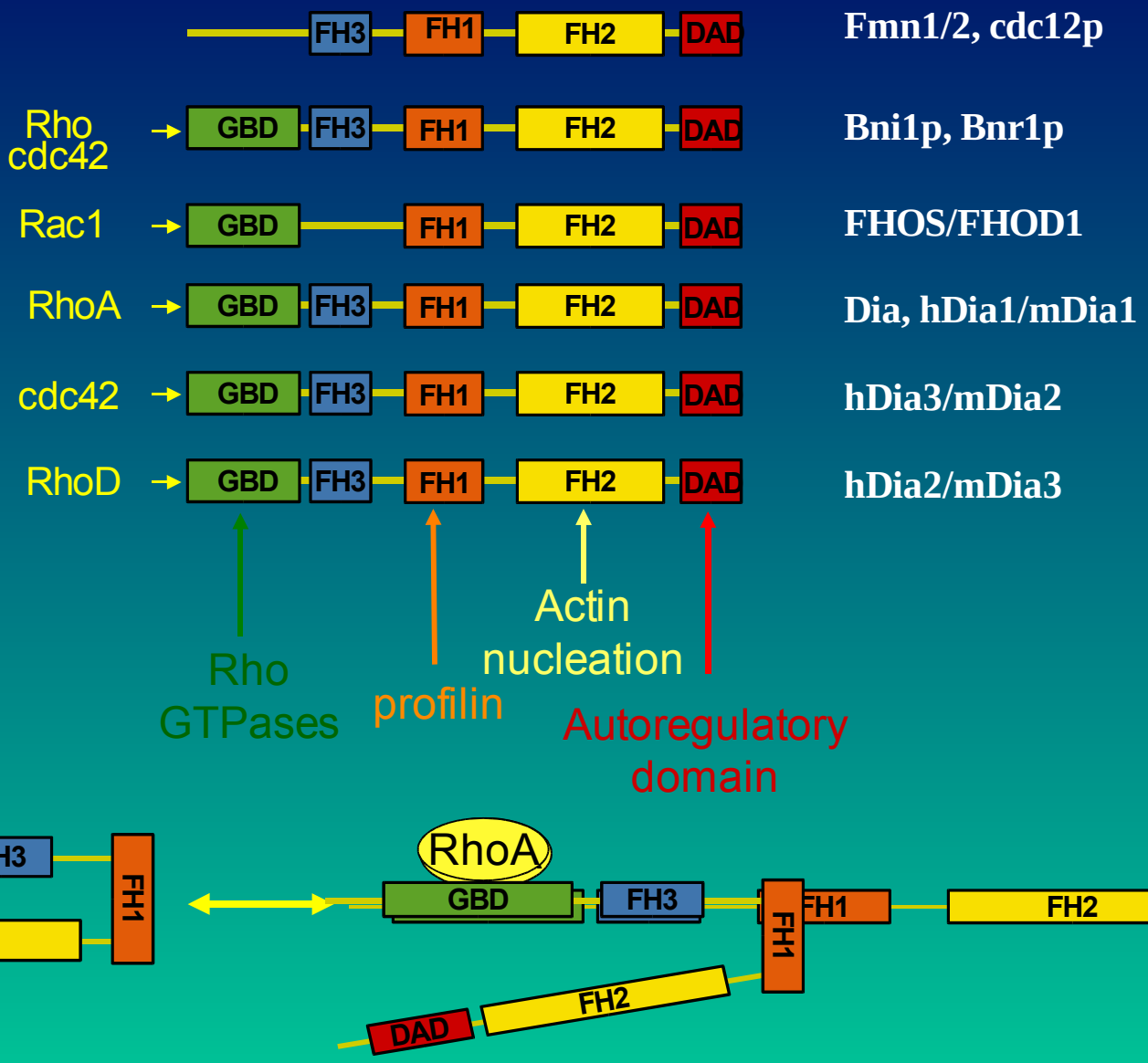
Branching nucleation (WASP/Arp2/3) processive growth



Barbed end and (formins)



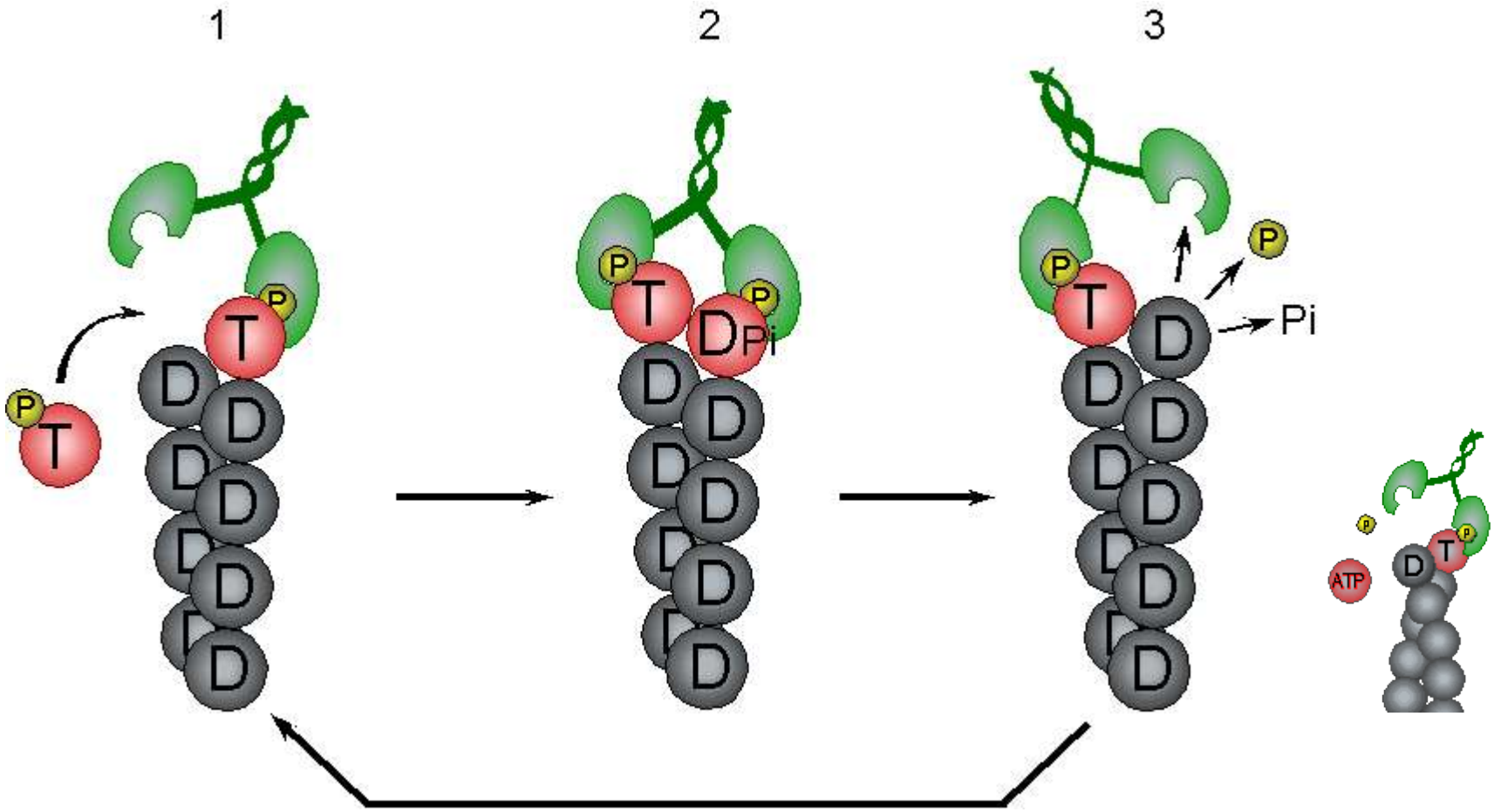
The formin family



Properties of formins

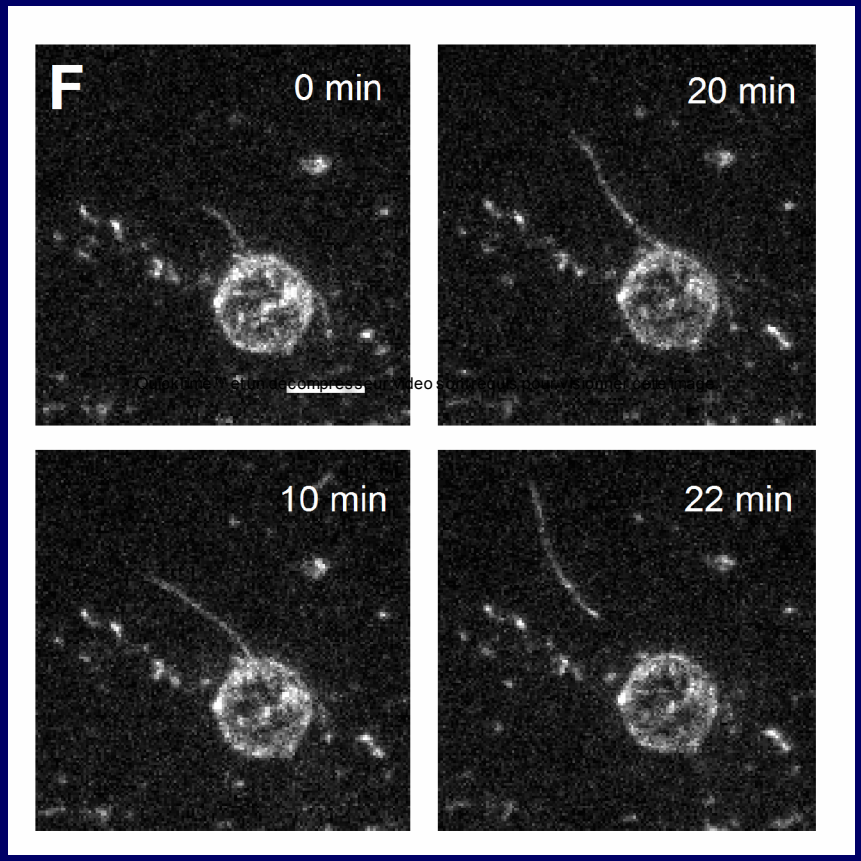
- Nucleate actin assembly (FH2 is sufficient)
- Active as FH2 or FH1-FH2 dimers
- Bind to barbed ends without greatly affecting rat parameters for actin assembly and disassembly
- Postulated to be processive « leaky cappers » remaining bound to growing barbed ends

Formin is a processive motor that directs barbed end assembly of actin filaments from profilin-actin

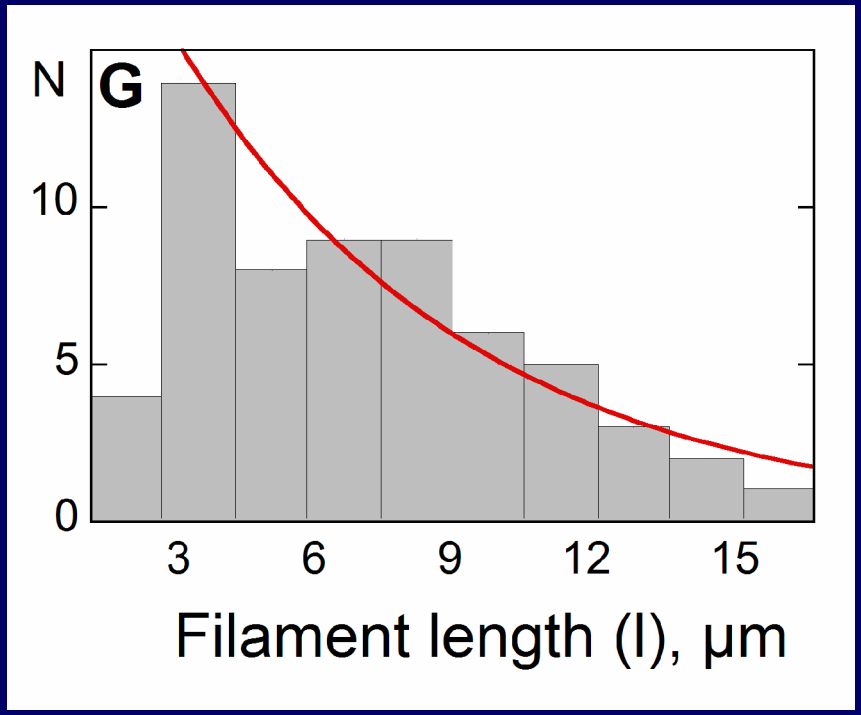


Romero et al., Cell (2004)

Formin remains bound to a growing barbed end for 1200 to 2500 seconds before detaching *(Romero et al., Cell, 2004)*

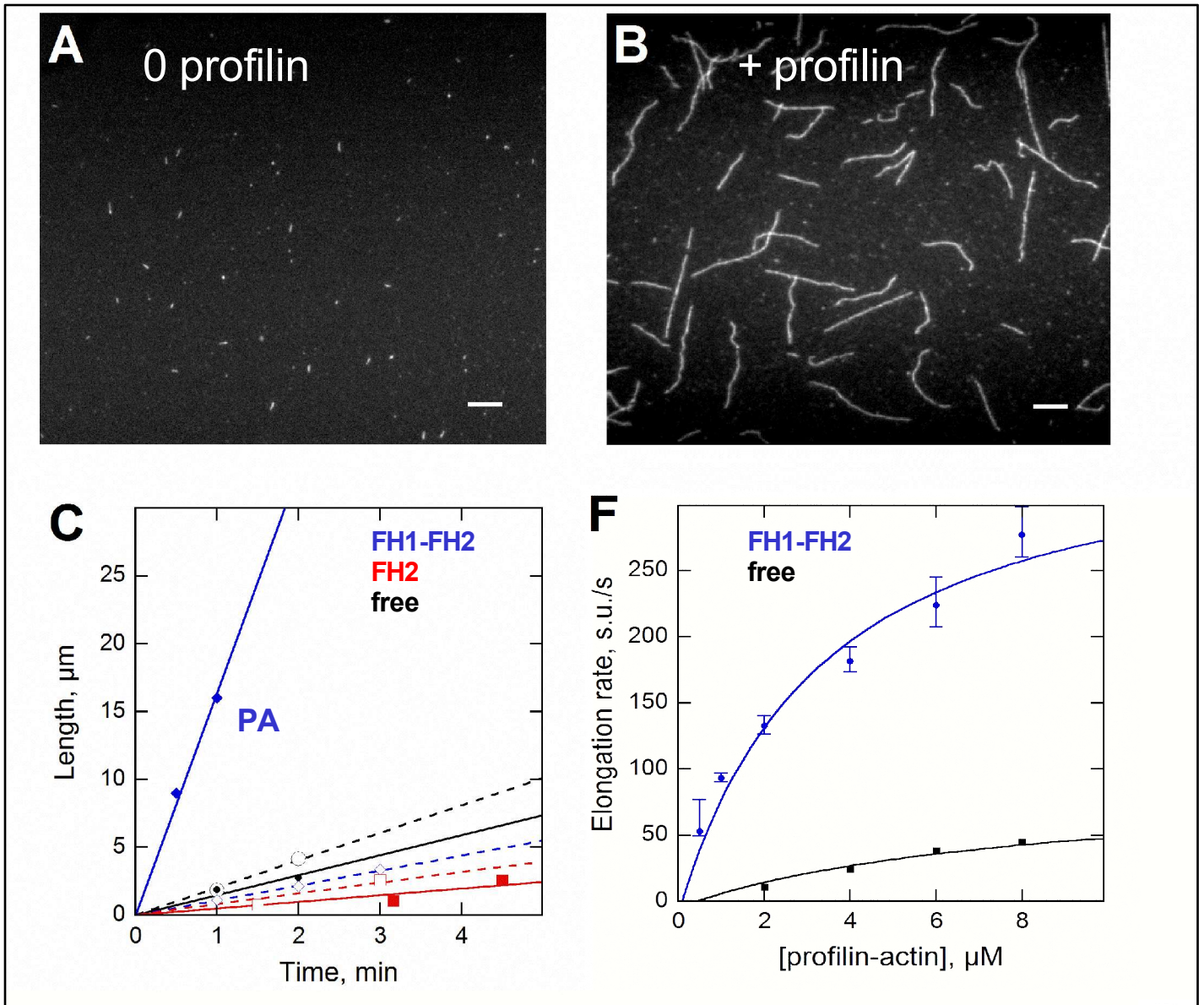


Solution of F-actin (0.5 µM) and profilin (4 µM)

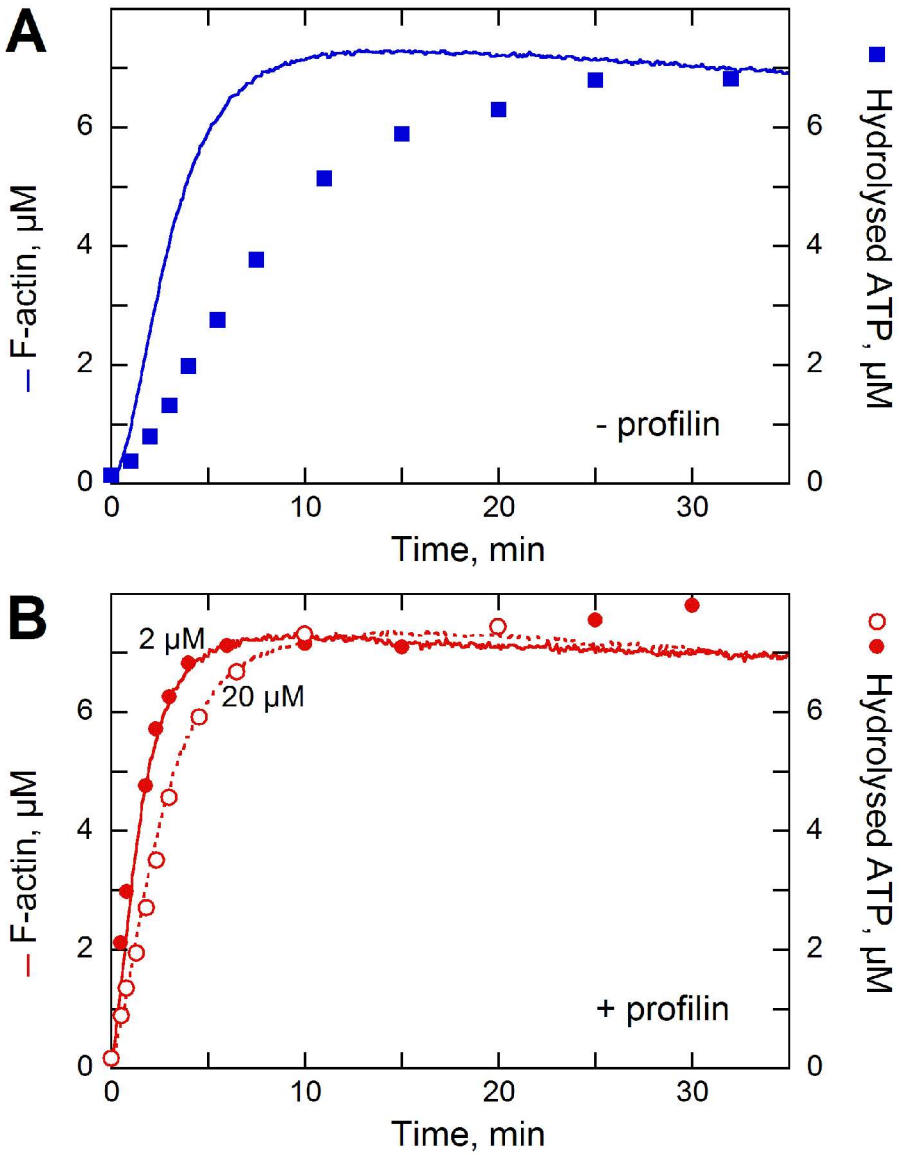


Frequency of detachment of a barbed end:
kd = 7.5. 10^{-4} ± 1.5.10-4 s^{-1}

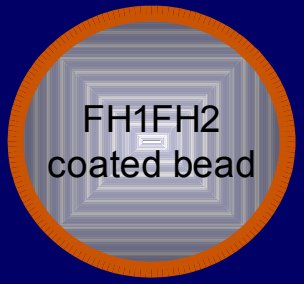
Formin increases the rate of profilin-actin association to barbed ends



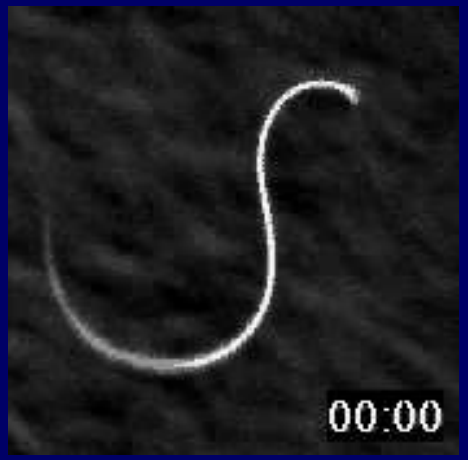
Formin increases the rate of ATP hydrolysis in profilin-actin assembly



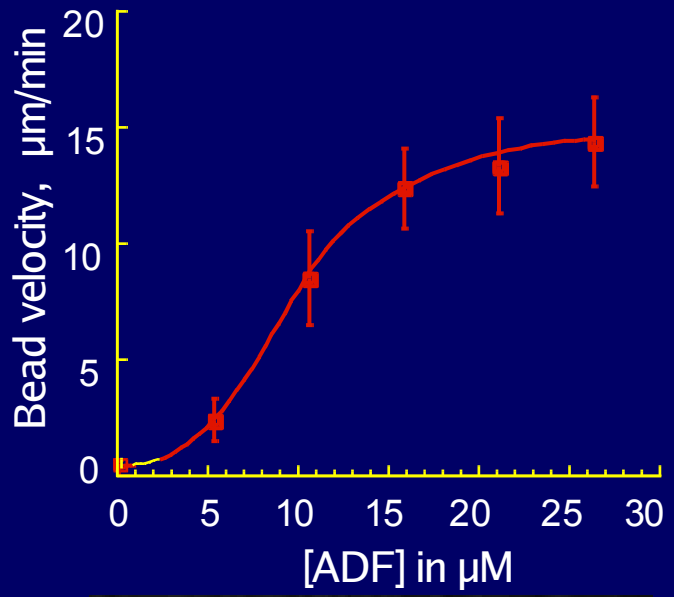
Reconstitution of formin-based motility

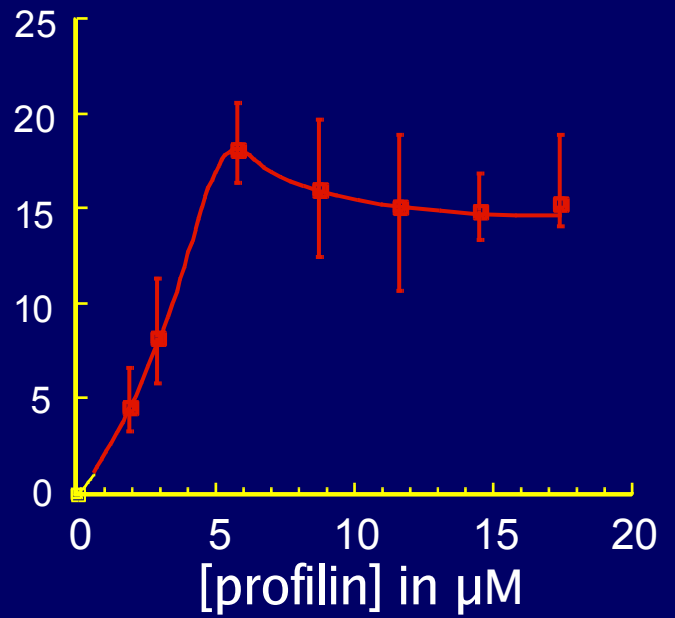


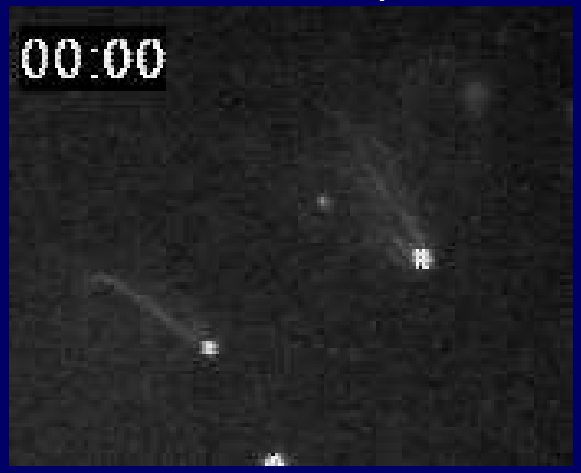
ADF : 4µM
Prof : 3µM
F-actin : 7µM



V = 2,5 µm/min







Actin-based motility

LEBS, CNRS, Gif-sur-Yvette

- Dominique Pantaloni
- Marie-France Carlier
- Emmanuèle Helfer
- Dominique Didry
- Diep Lê

Stanislav Samarin

Sebastian Wiesner

Christophe Le Clainche

- Stéphane Romero
- Vincent Delatour

Collaborators

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Coumaran Egile

Philippe Sansonetti

(Institut Curie):

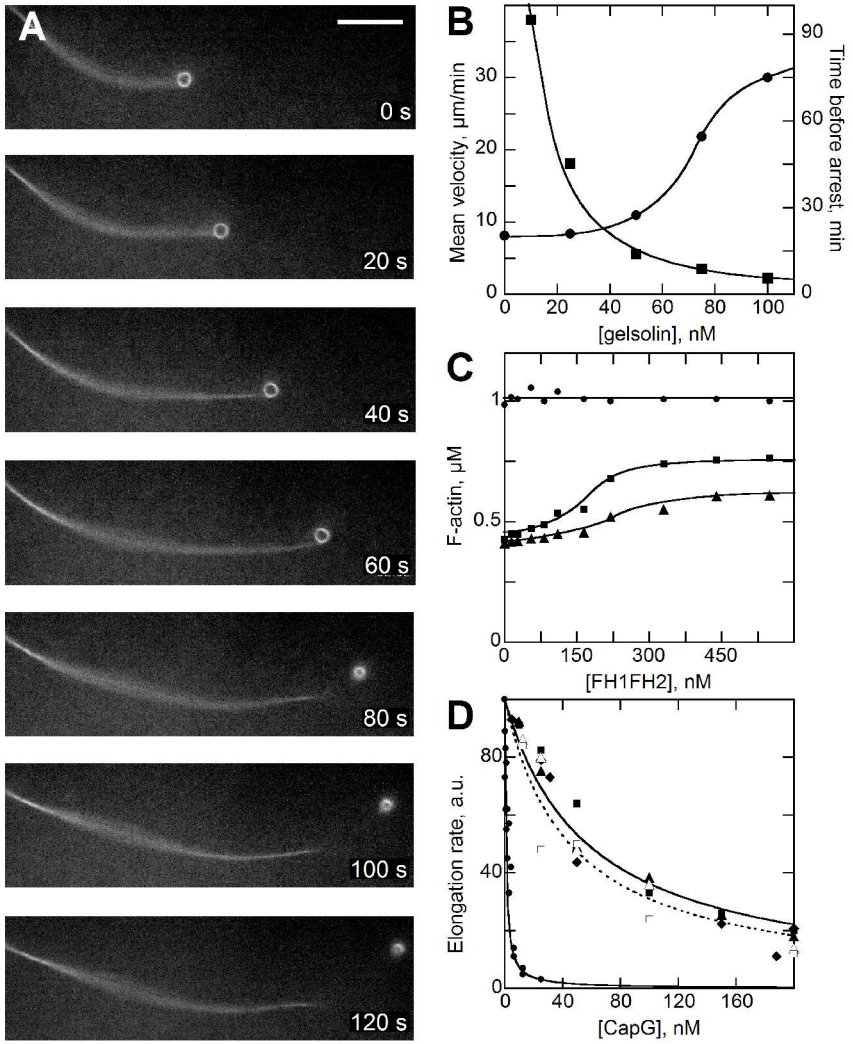
Jacques Prost

Cécile Sykes

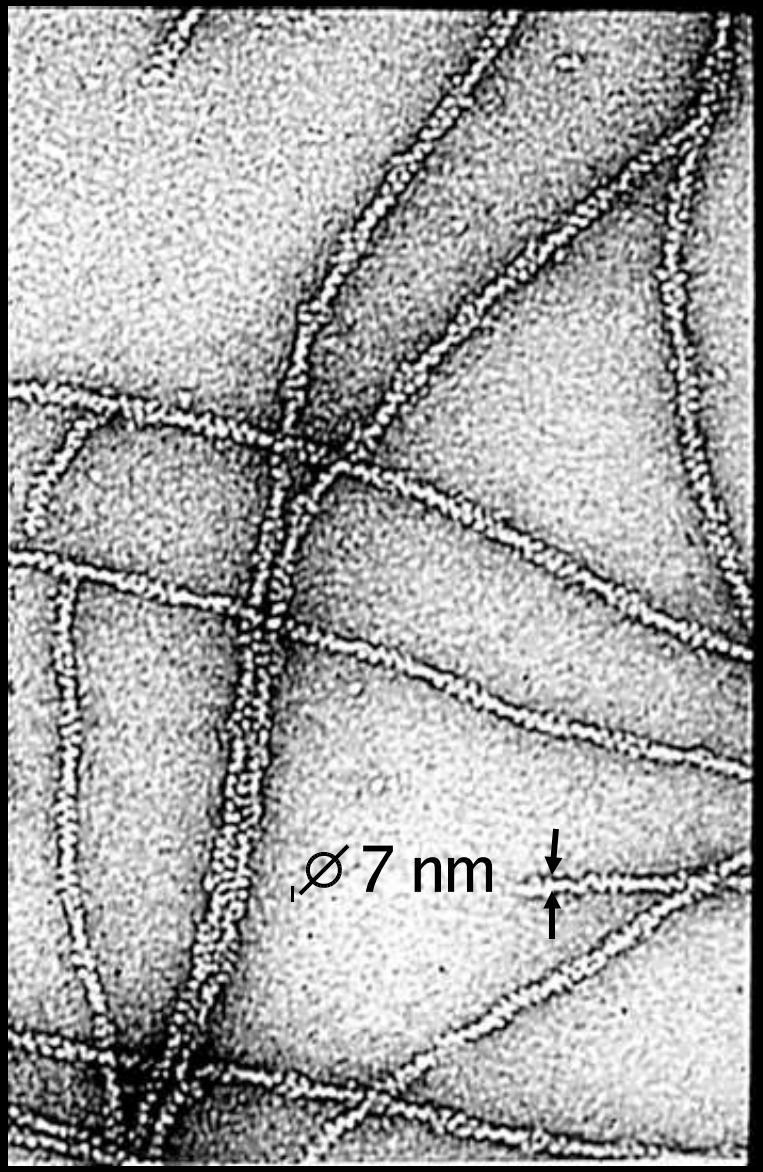
(Harvard medical school) :

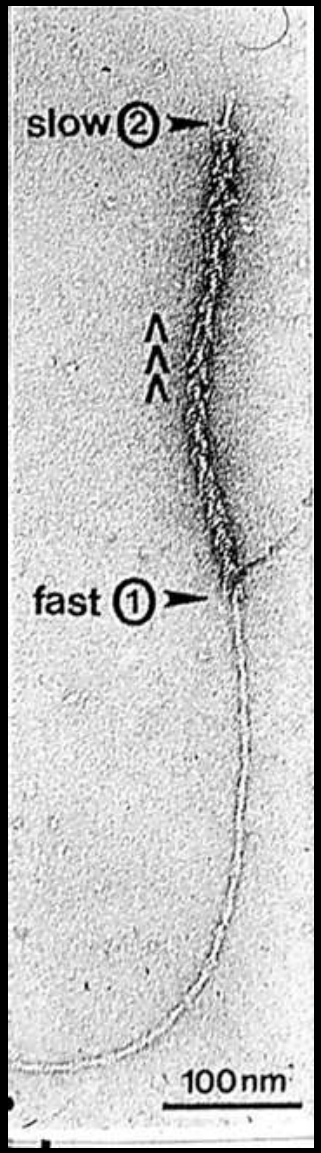
Christine Kocks

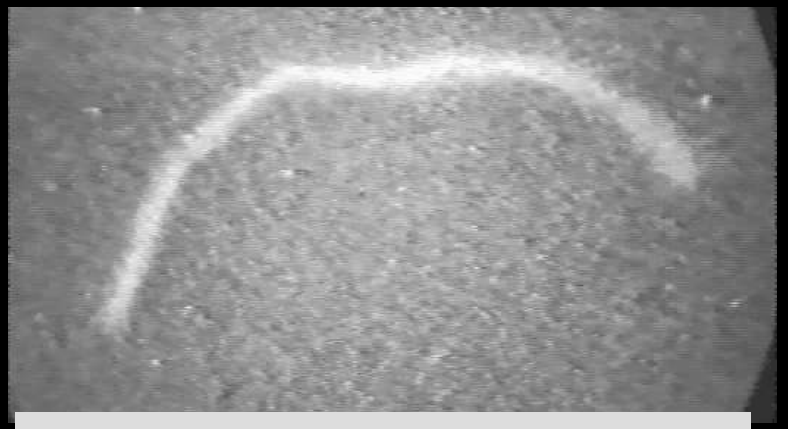
Capping proteins regulate the speed and duration of formin-based motile processes



Microfilaments: Polarity, Flexibility.

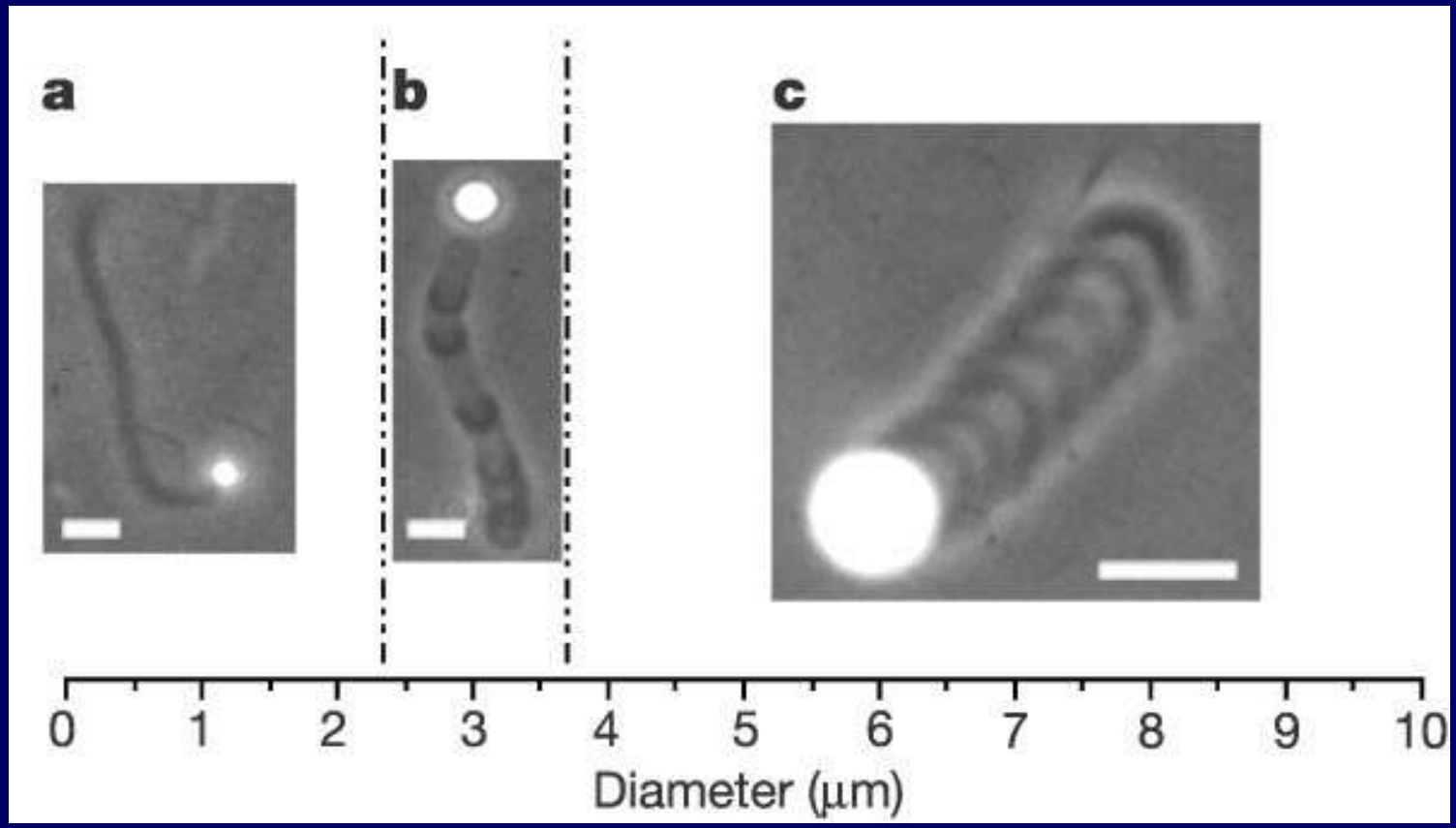




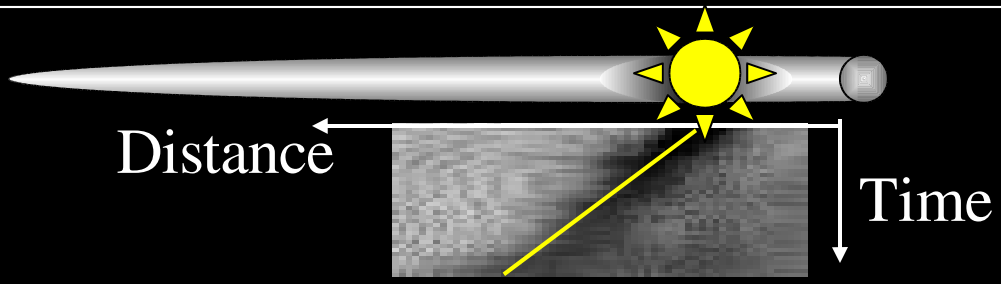


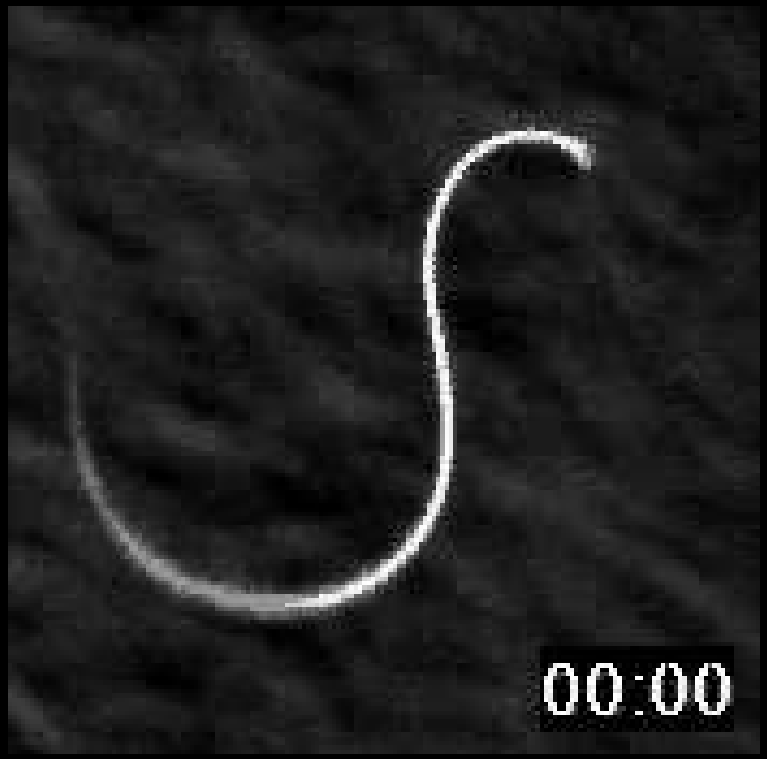
Persistance length : 12 µm

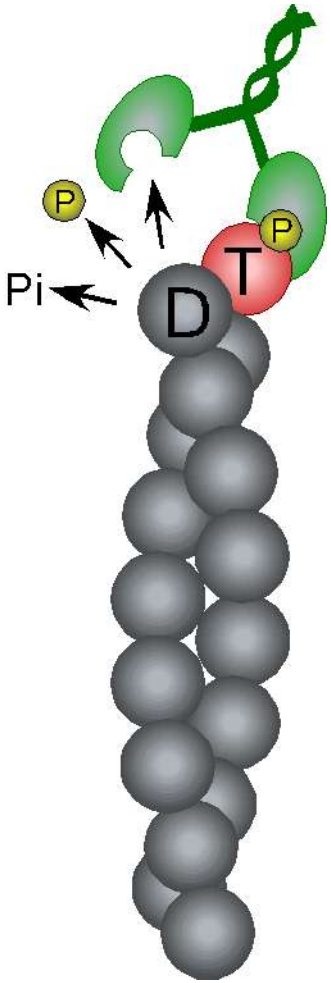
Mimicking « hopping *Listeria* »: From continuous to periodic actin-based movement



Formin drives rapid site-directed barbed end assembly of actin filaments from profilin-actin

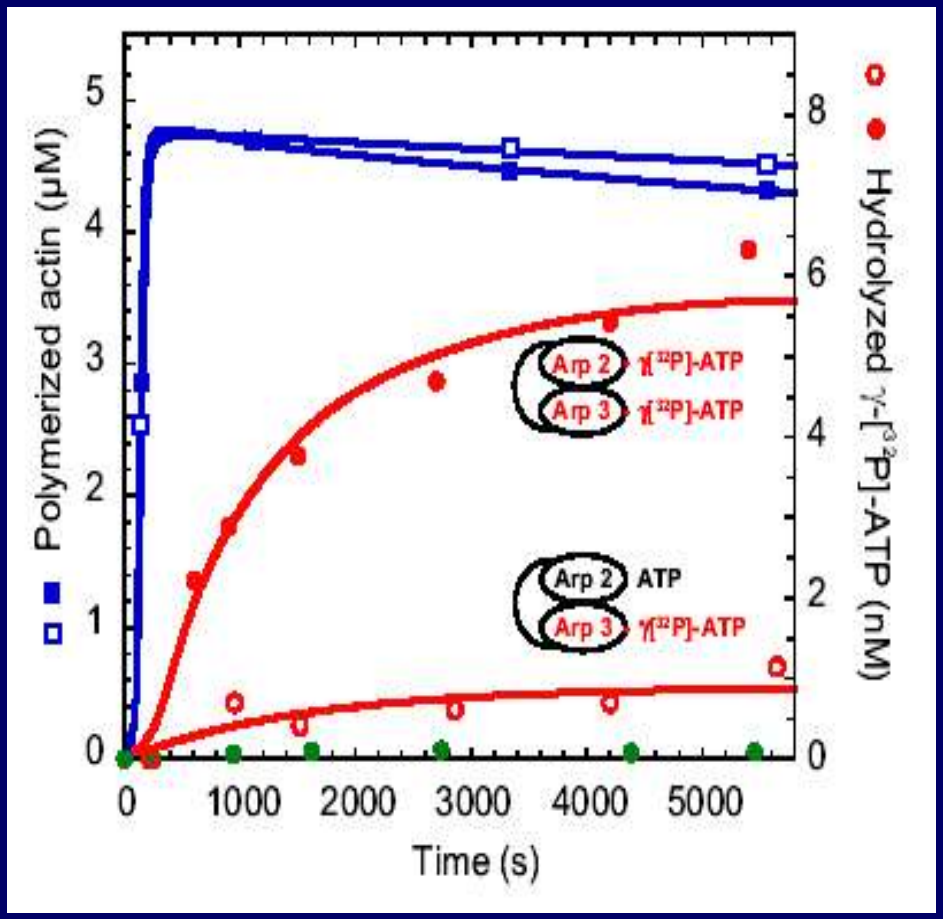


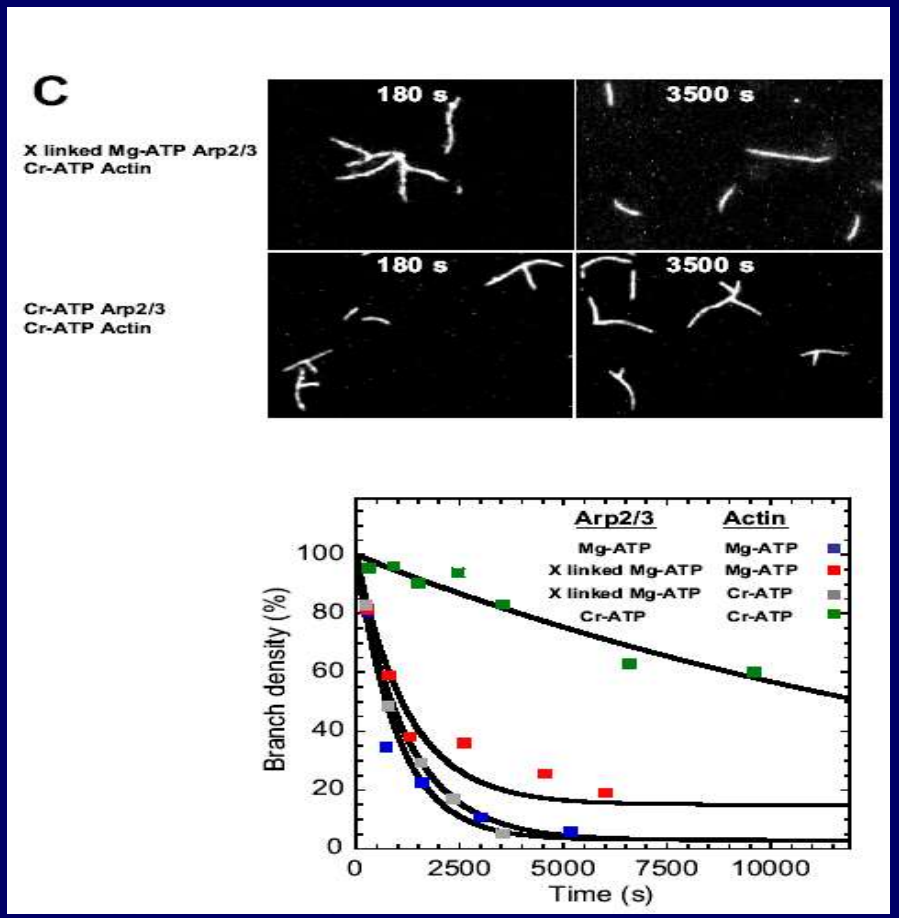



P
P
Pi
T
D

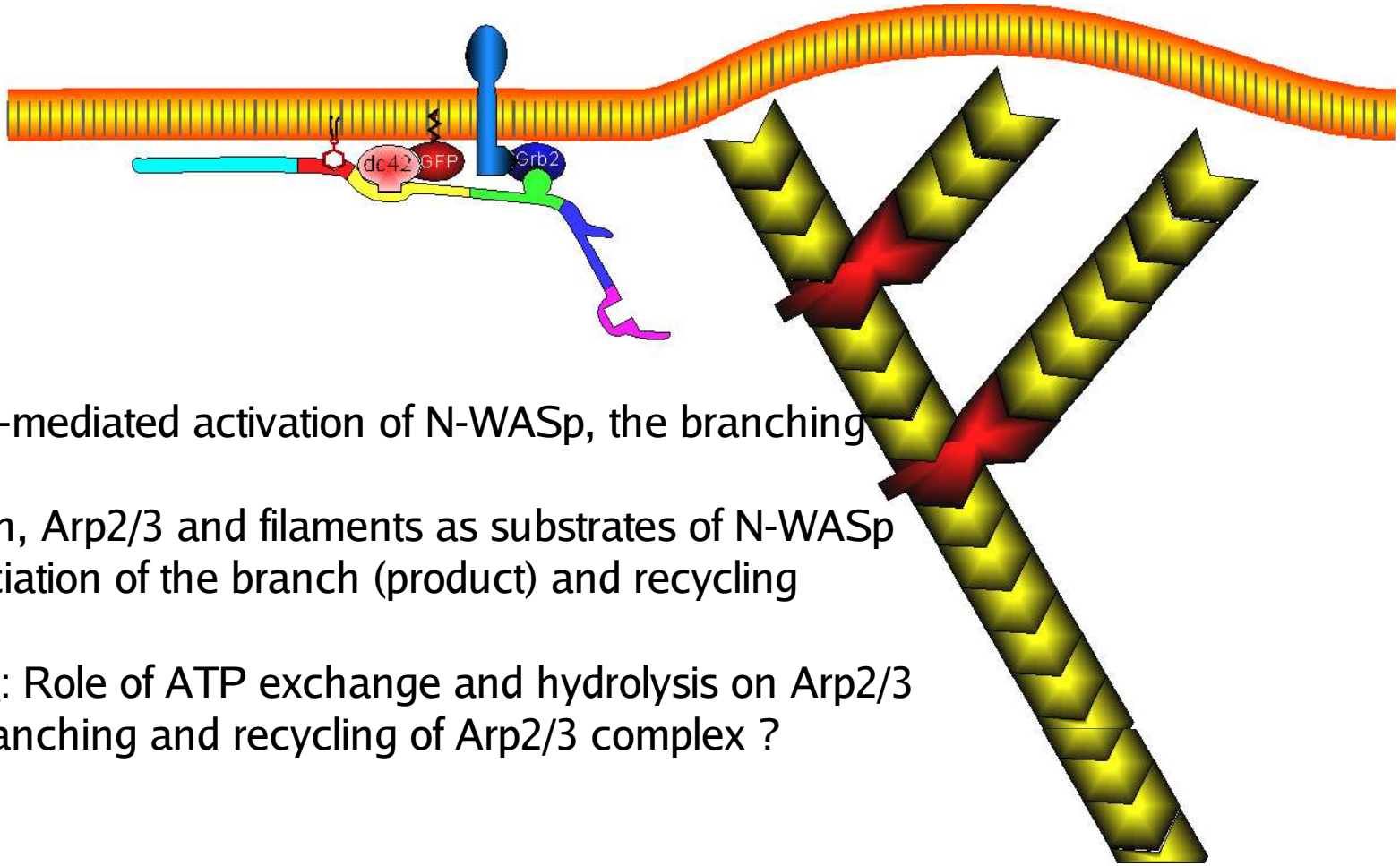
ATP hydrolysis occurs on Arp2 only following branch formation and drives debranching

(Le Clainche » et al., 2003, revised, PNAS)





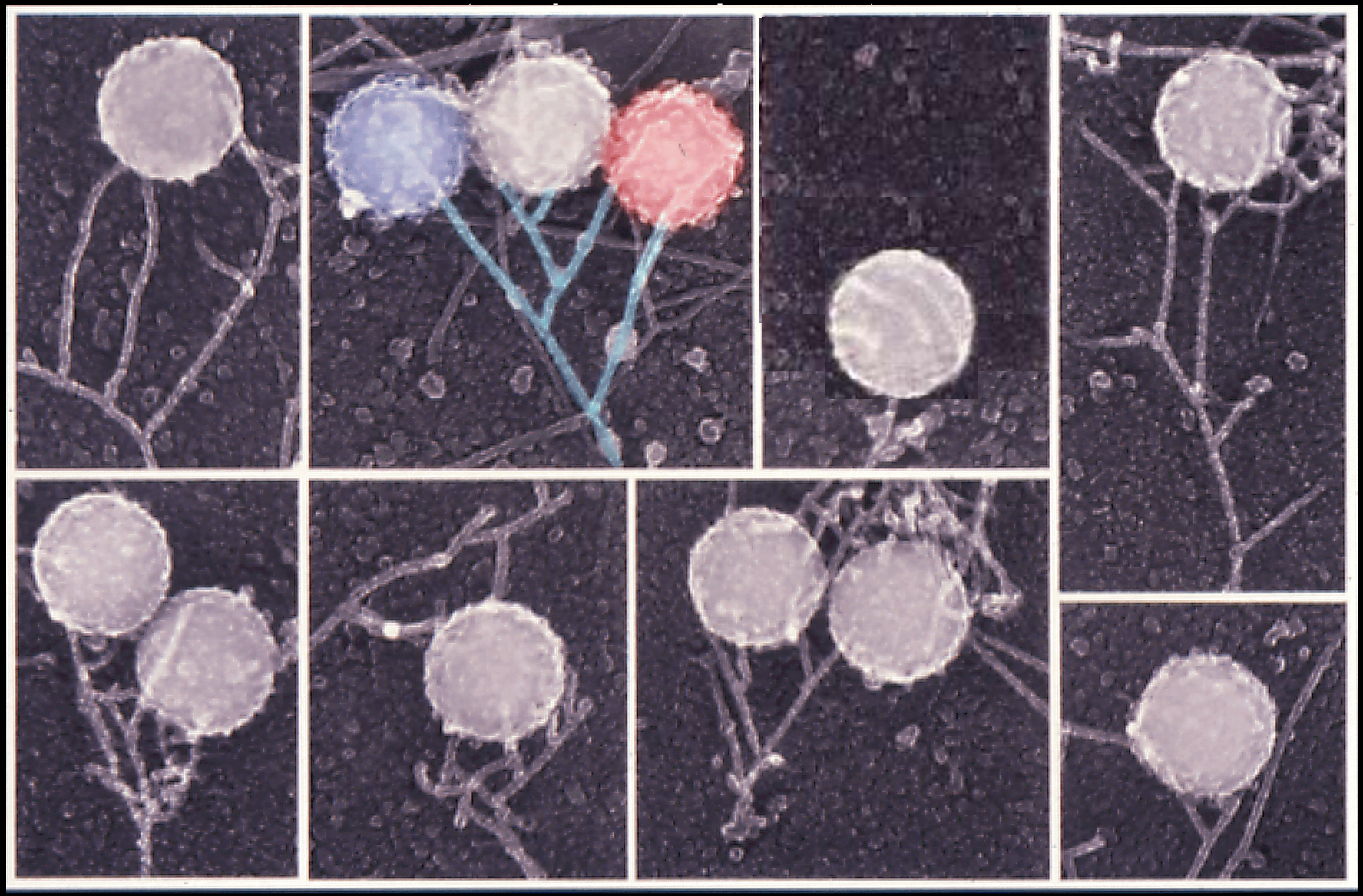
Model for actin-based motility



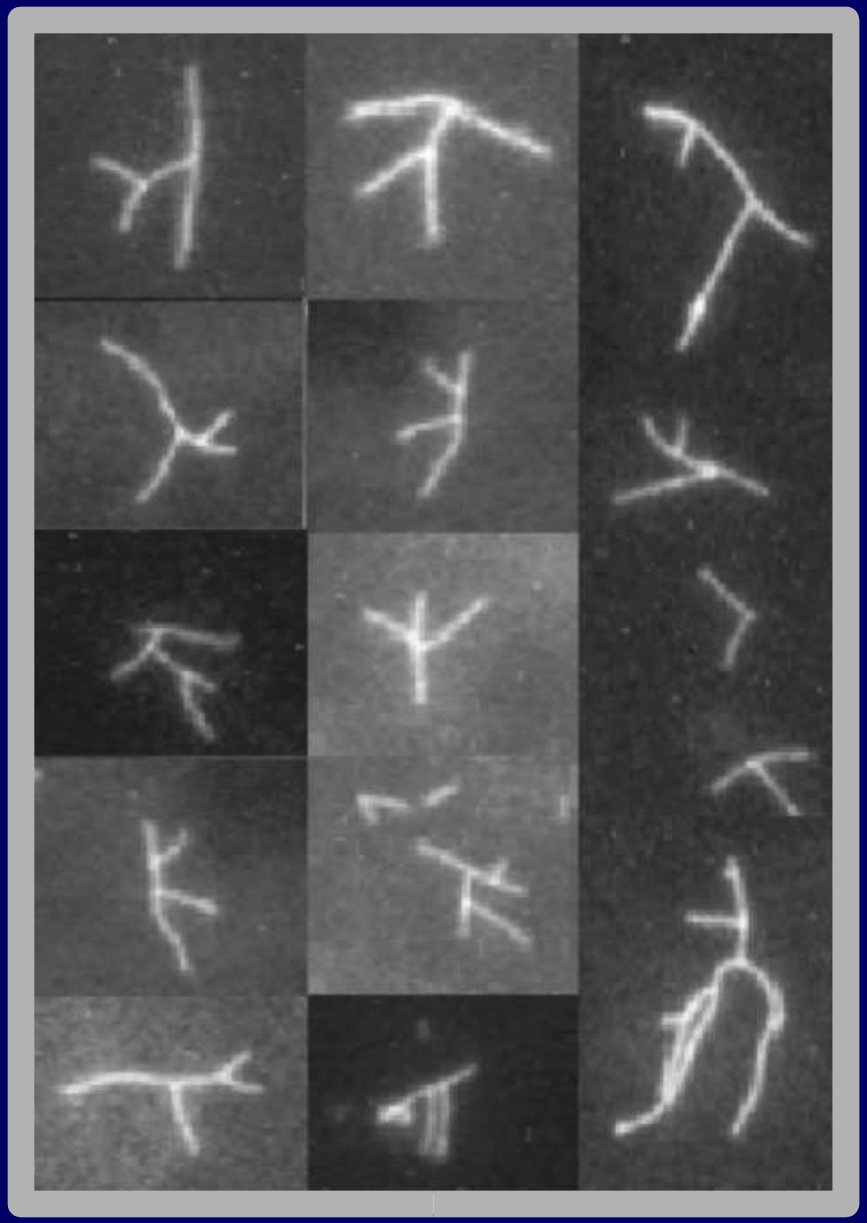
1. Signal-mediated activation of N-WASp, the branching enzyme
2. G-actin, Arp2/3 and filaments as substrates of N-WASp
3. Dissociation of the branch (product) and recycling

Question: Role of ATP exchange and hydrolysis on Arp2/3 in the branching and recycling of Arp2/3 complex ?

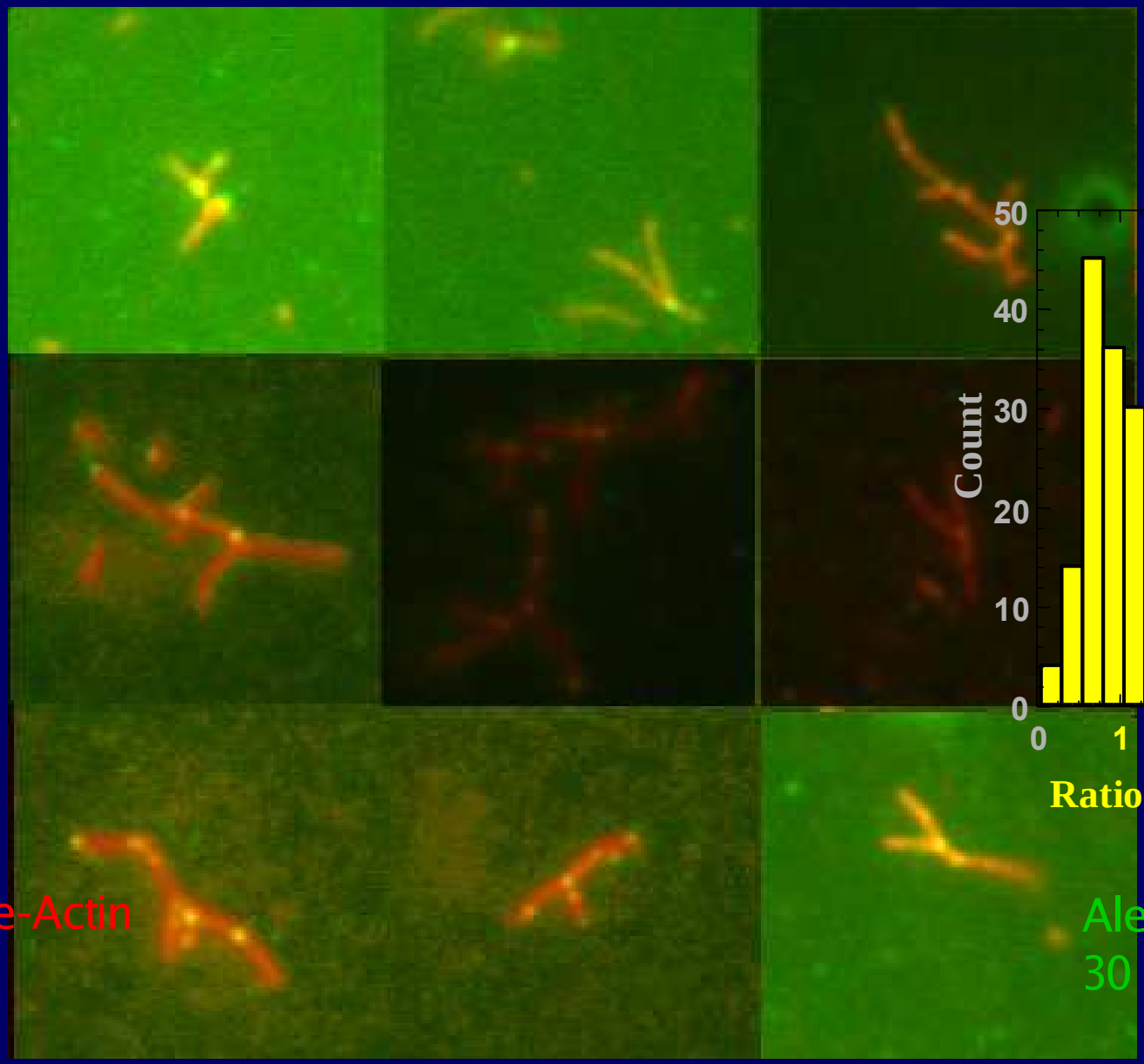
Dendritic nucleation on ActA beads



Gallery



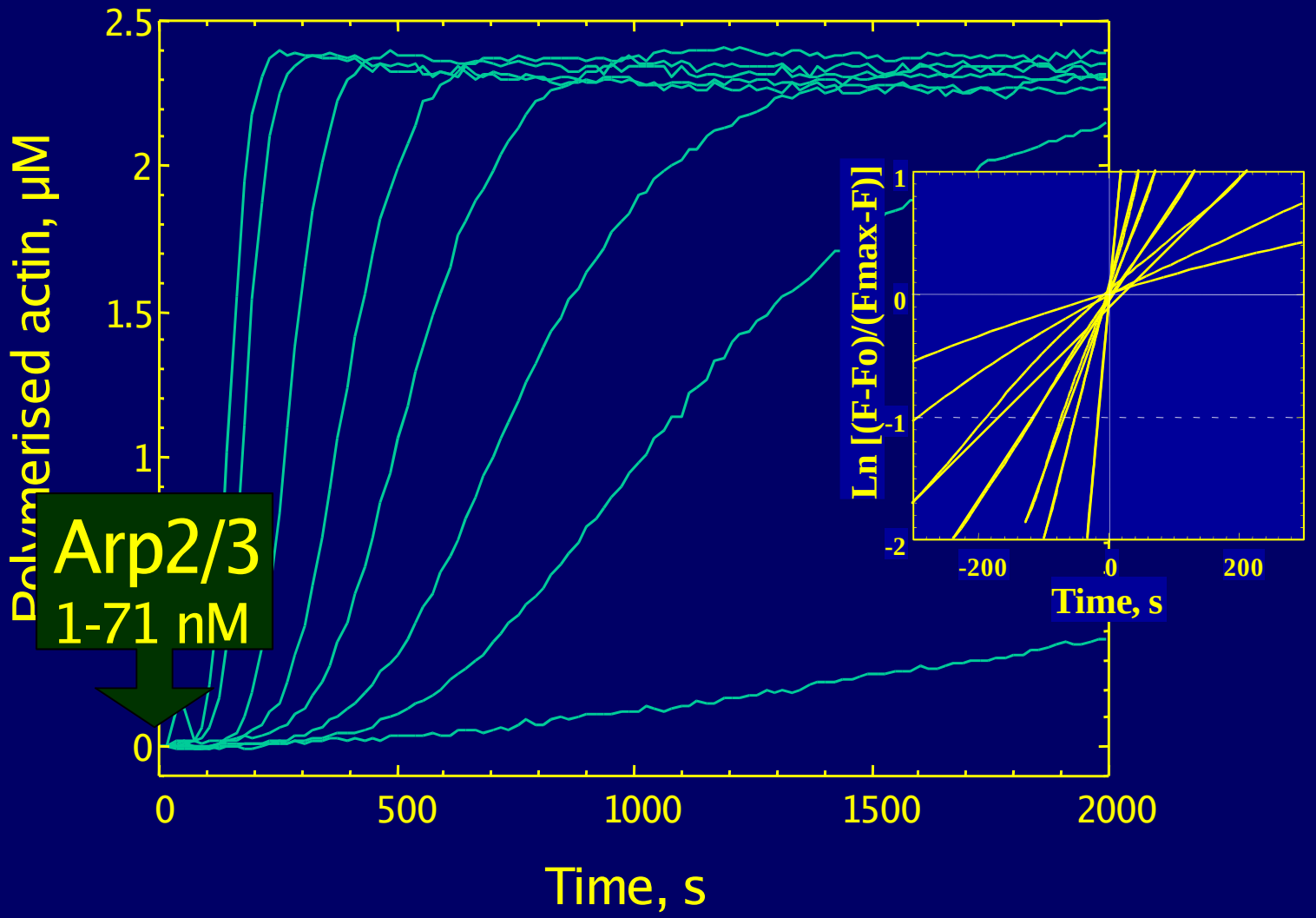
Localization of Arp2/3 complex at the branch junction and on the mother filament



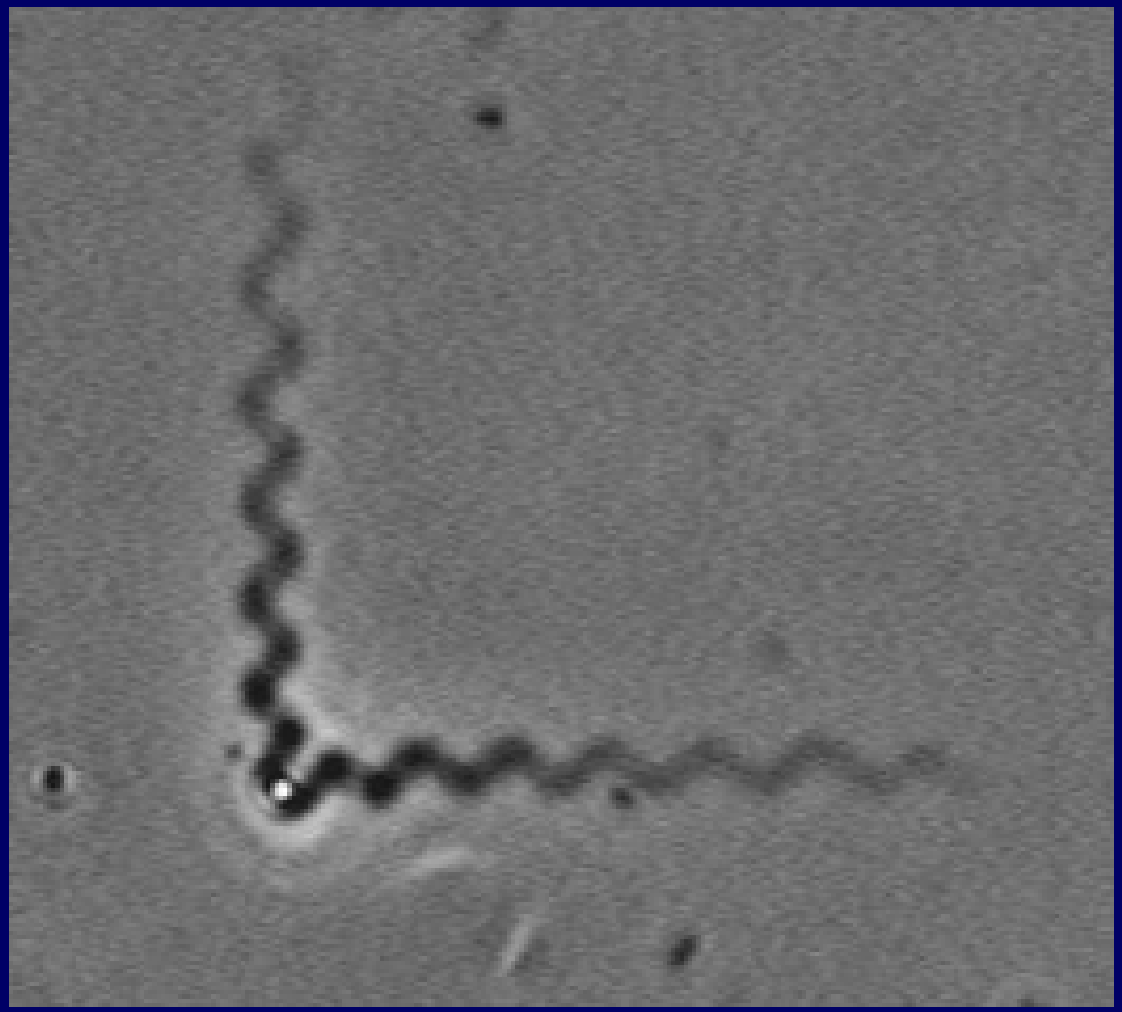
Rhodamine-Actin
200 ms

Alexa488-Arp2/3
30 seconds

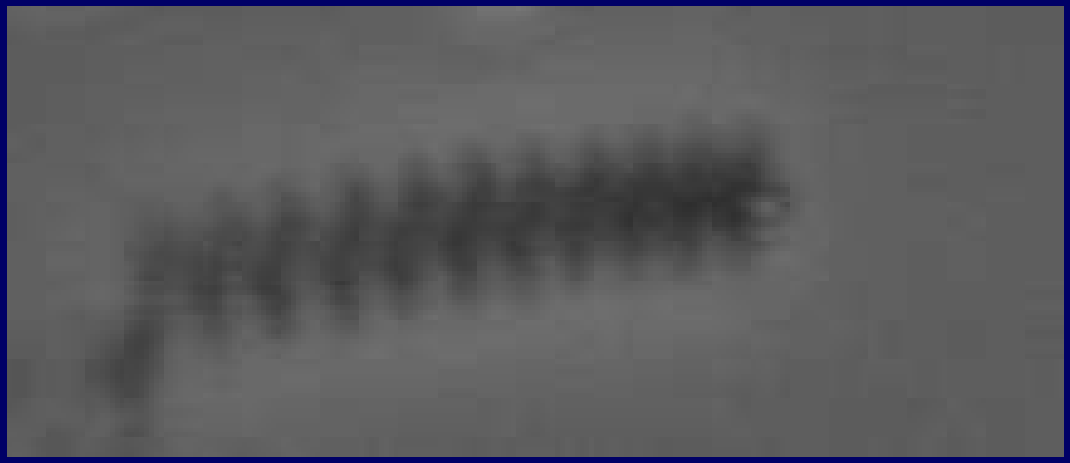
Arp2/3-stimulated actin polymerization is an autocatalytic process

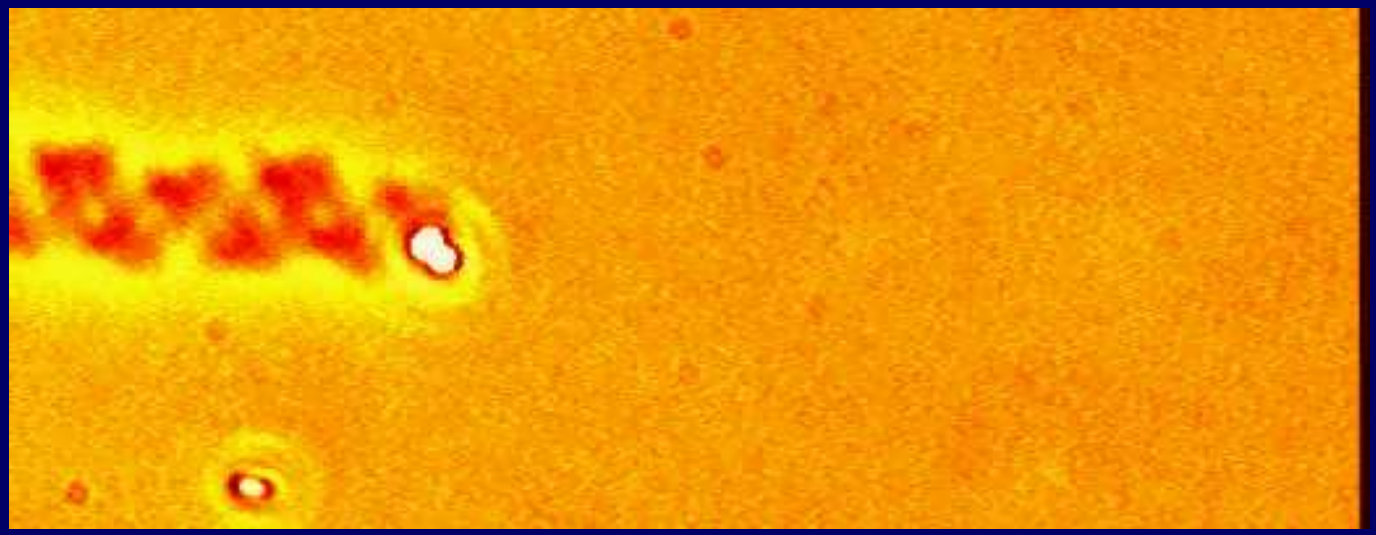


Contraintes de Polymérisation



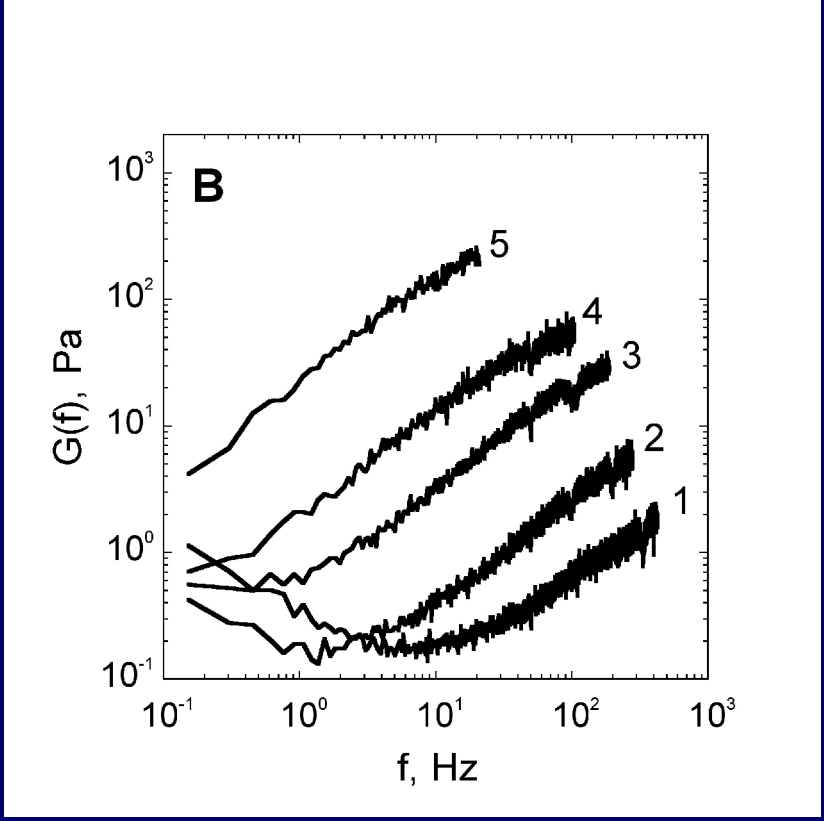
Comète Hélicoïdale



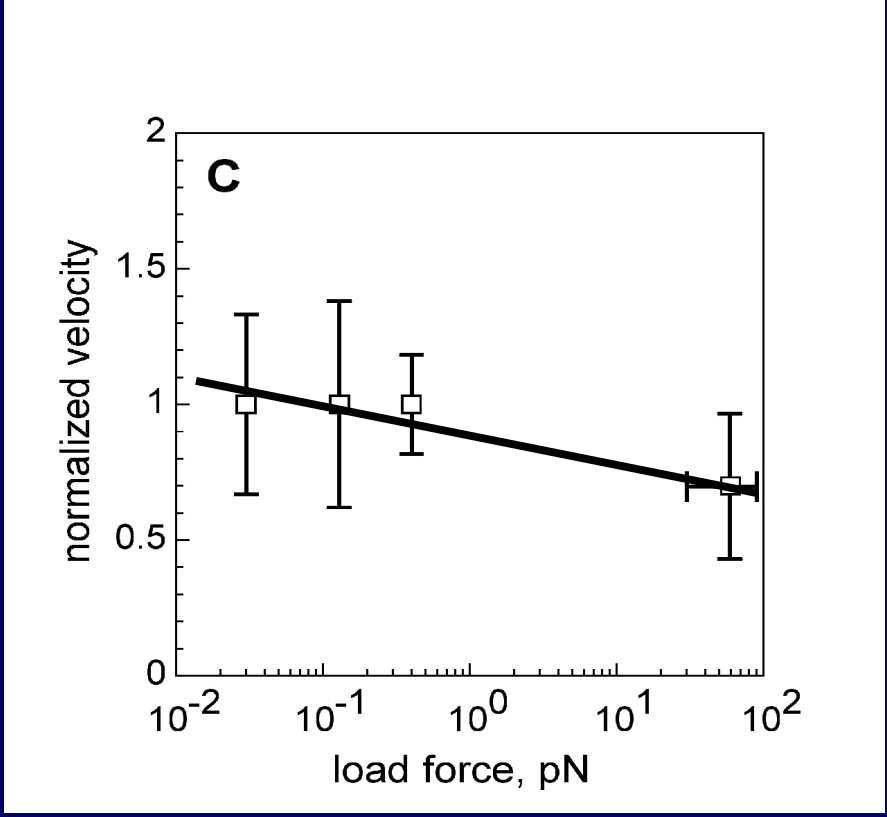


Forces of the order of 1 nN are developed by actin polymerization

Effect of methylcellulose on G(f)
(Laser Tracking Microrheology)



Force-velocity relationship



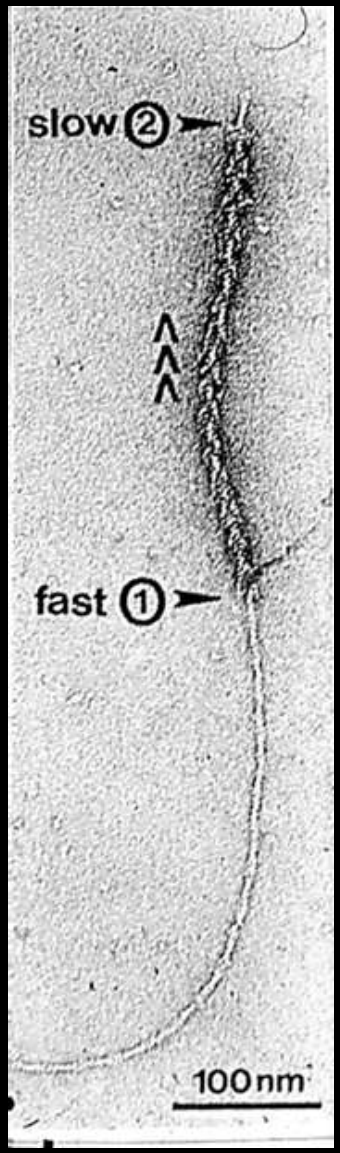
Using the motility assay to understand the mechanism of production of force and directional movement

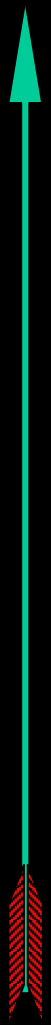
- Control of the concentration of soluble proteins in the motility medium.
- Control of the surface density of filament branching enzyme (N-WASP or ActA).
- Load/velocity relationship: control of the size of the bead and of the viscosity of the medium.
- Frequency of filament branching during movement: two fluorophores (actin and Arp2/3)

Actin-based motility:

How vectorial assembly of actin filaments can generate force and movement

Microfilaments: Polarité, Flexibilité.

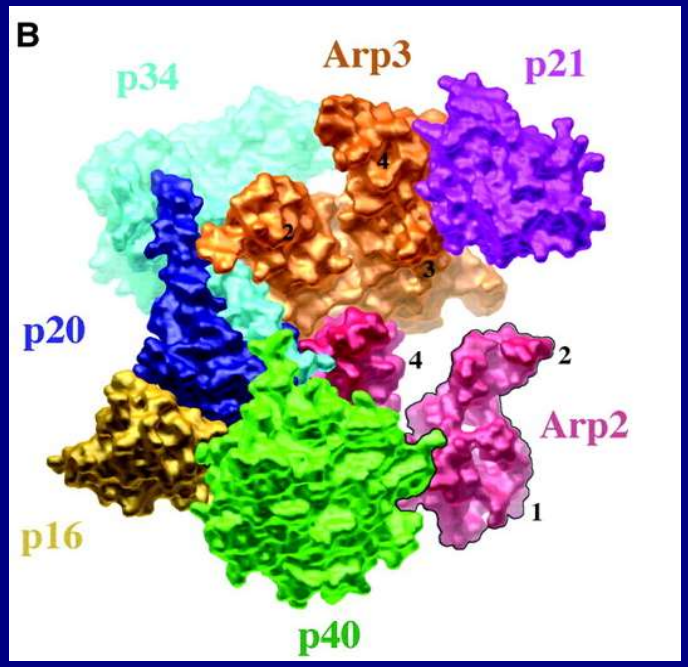




PERSPECTIVES

- Biomimetics: Reconstitution of lamellipodium protrusion (force applied to a membrane, functionalized liposome)
- Coupling of adhesion and protrusion during cell migration: concerted actin dynamics at focal contacts and in lamellipodium.
- Signaling, actin-based motility and morphogenesis: specifying different motile actin-based structures.

Arp2/3 Complex : downstream target of multiple signaling pathways leading to actin assembly



R. Robinson et al (Science, 2001)

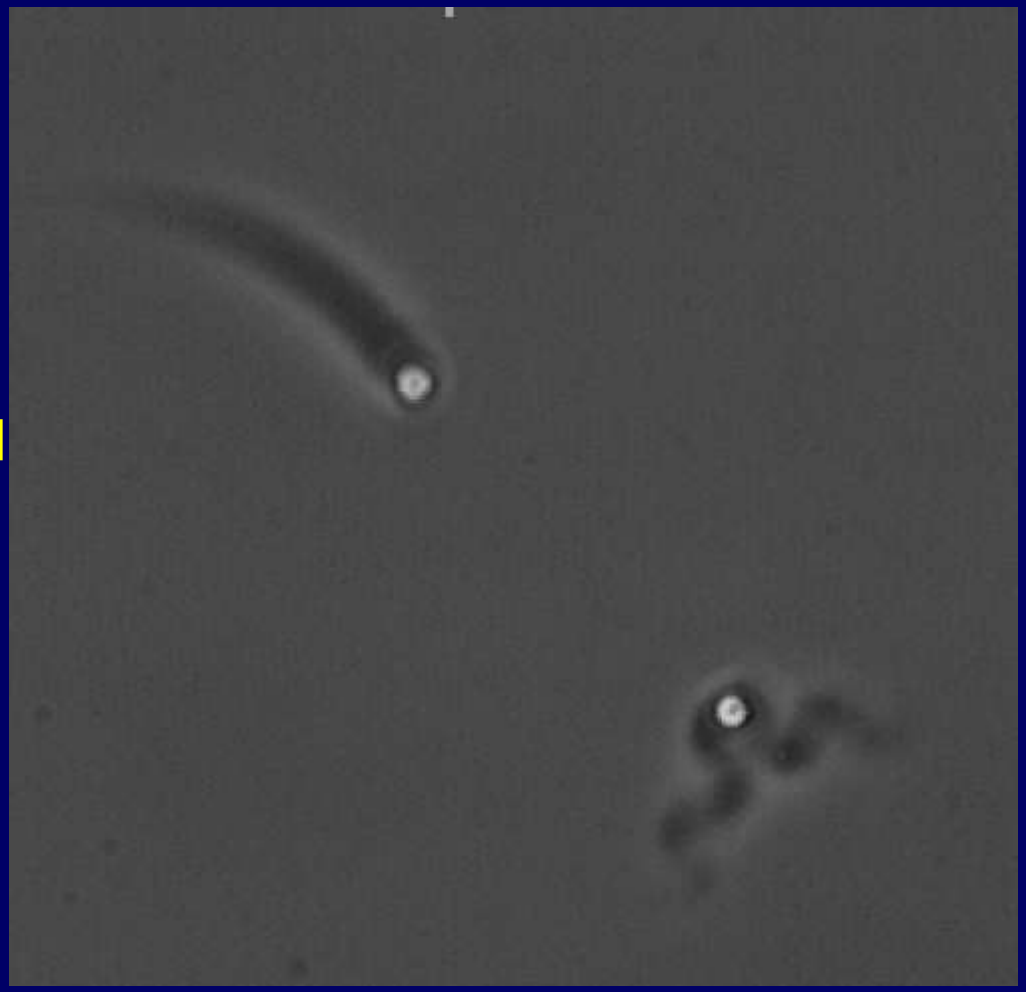
- **Localized in actin-based motility processes:**
  - *Listeria* and *Shigella* propulsion (Welch, 1997; Egile et al., 1998)
  - Lamellipodium extension (Svitkina and Borisy, 1999)
  - Phagocytic cups (Machesky, 2000)
  - Endocytic vesicles (Taunton et al., 1999)
  - Actin patches (Li 1997; Cooper 1999)
  - Cadherin-mediated adhesion (Yap, 2002)
  - Morphological events in *Drosophila* (Cooley, 2002)
- **Must interact with an activator:**
  - ActA on *Listeria*
  - WASP and Scar/WAVE proteins in

03:18

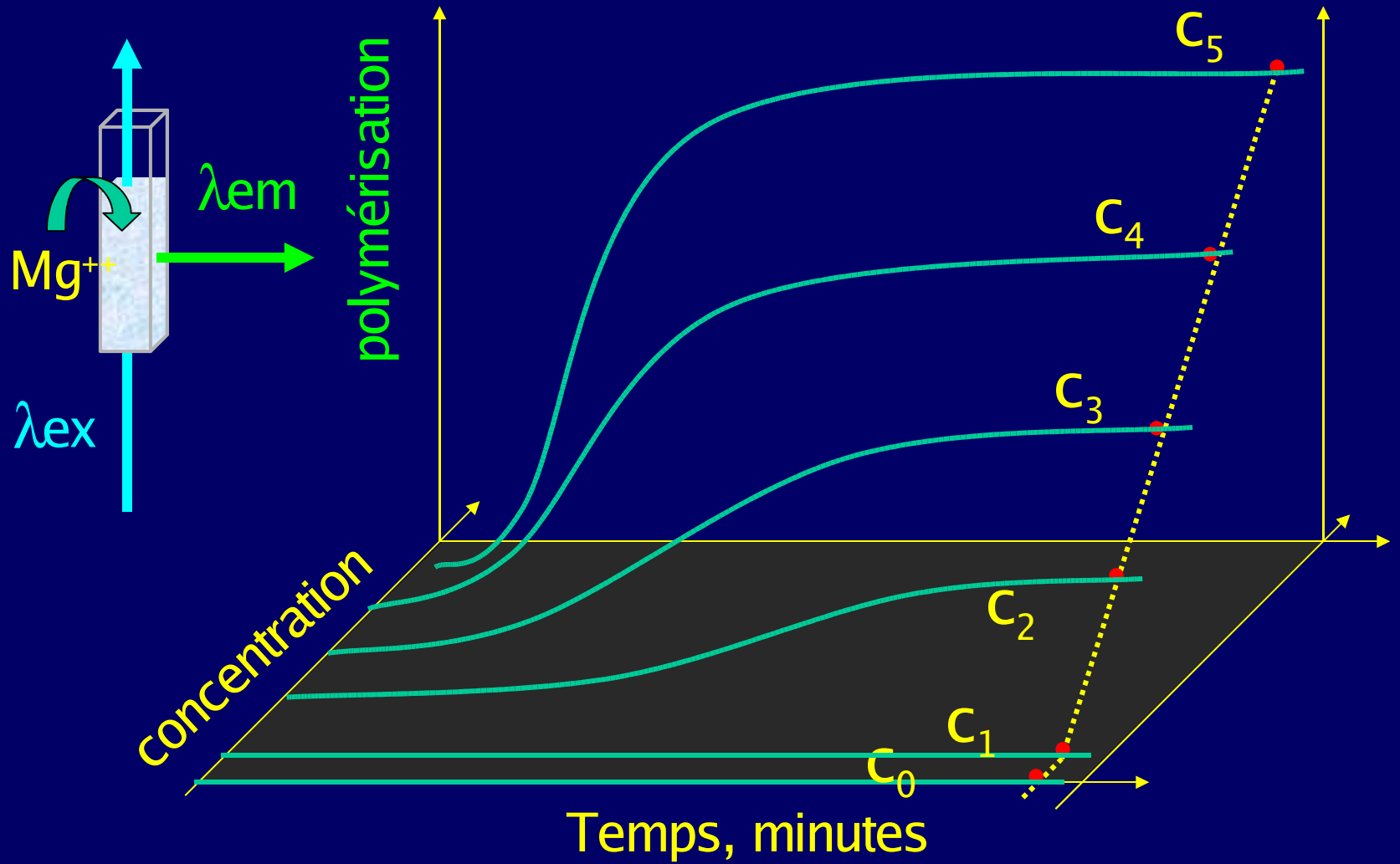
Encounters of the third kind

Motility medium :

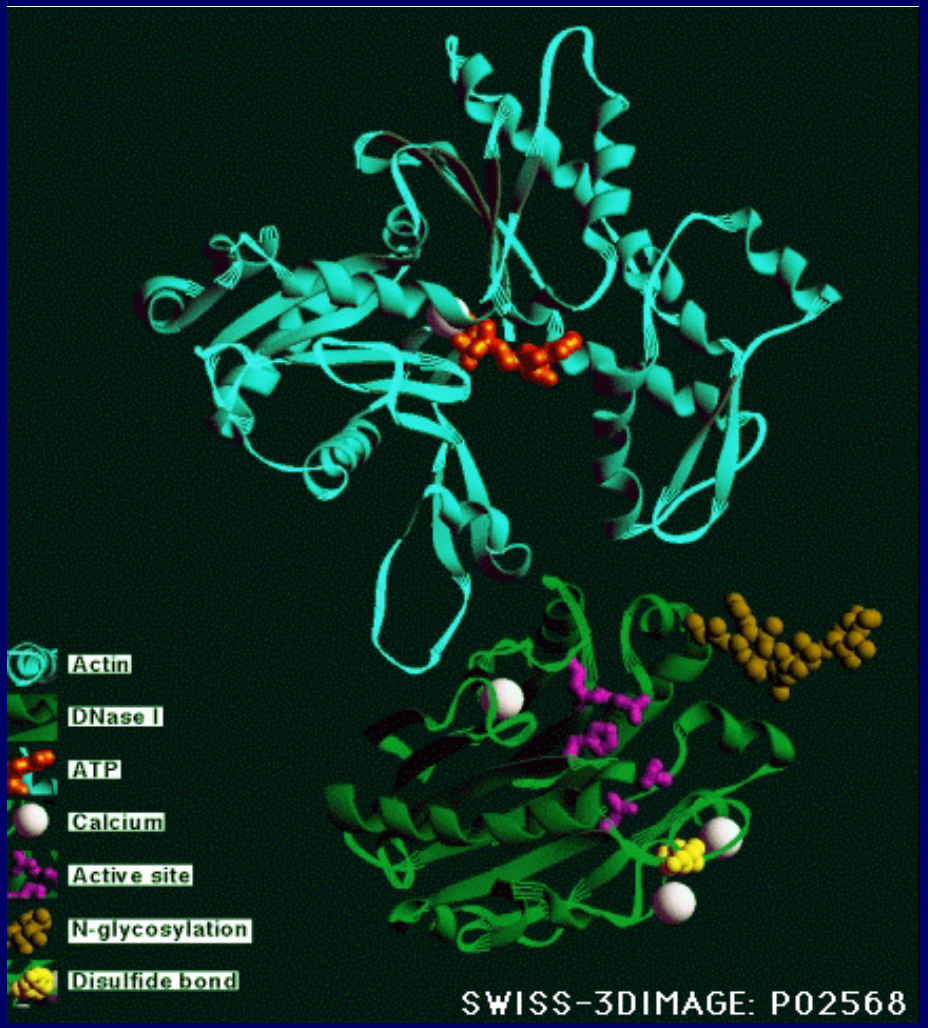
N-WASP	bead-bound
Arp2/3	0.1 μM
Capping Protein	0.1 μM
ADF	2 μM
ATP-actin+F-actin	8 μM
Profilin	2 μM



Nucléation Polymérisation : f(c)



Structure de l'Actine



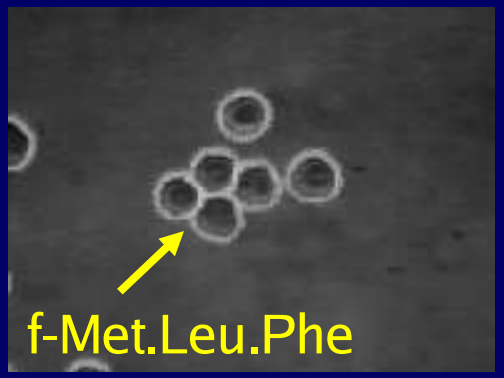
Séquence des 375 acides aminés constituant l'actine humaine

MDDDIAALVVDNGSGMCKAGFAGDDAP
RAVFPSIVGRPRHQGVMVGMGQKD
SYVGDEAQSKRGILTLKYPIEHGIVTN
WDDMEKIWHHTFYNELRVAPEEHP
VLLTEAPLNPKANREKMTQIMFETFNT
PAMYVAIQAVLSLYASGRTTGIVM
DSGDGVTHTVPIYEGYALPHAILRLDL
AGRDLTDYLMKILTERGYSFTTTA
EREIVRDIKEKLCYVALDFEQEMATAA
SSSSLEKSYELPDGQVITIGNERF
RCPEALFQPSFLGMESCGIHETTFNSI
MKCDVDIRKDLYANTVLSGGTTMY
PGIADRMQKEITALAPSTMKIKIIAPP
ERKYSVWIGGSILASLSTFQQMWI
SKQEYDESGPSIVHRKCF

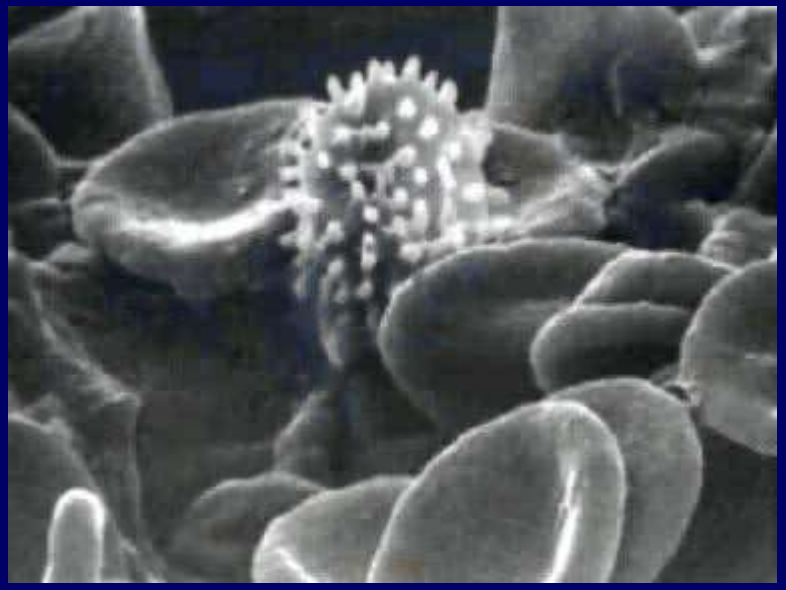
Actin filaments in cell movement and morphogenesis

- Actin filaments have a polar structure
- They are semi-flexible polymers
- Assembly dynamics is regulated *in vivo*
- Filament assembly is a dissipative reaction (hydolysis of actin-bound ATP)

Cell motility and signaling

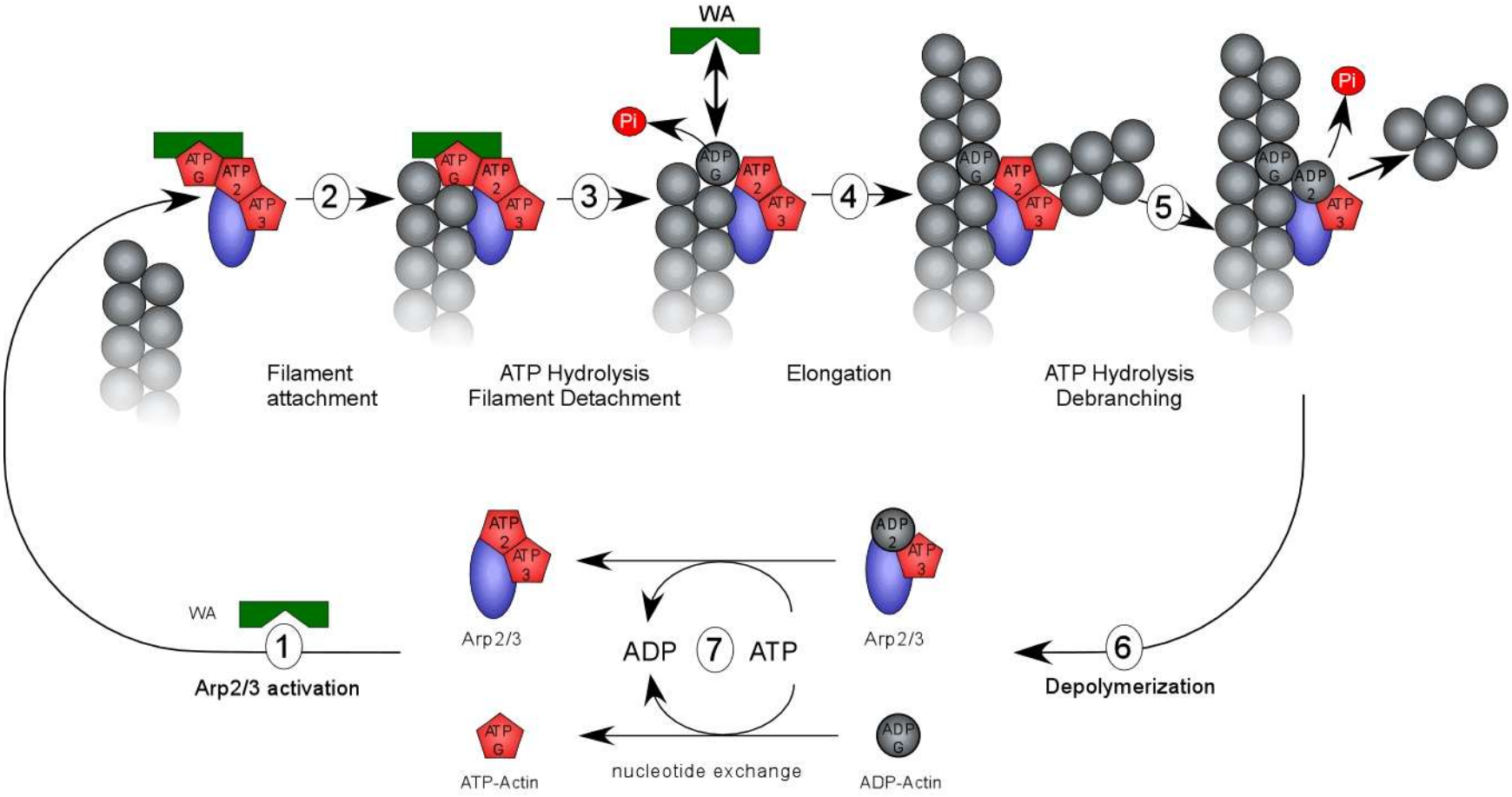


Neutrophils



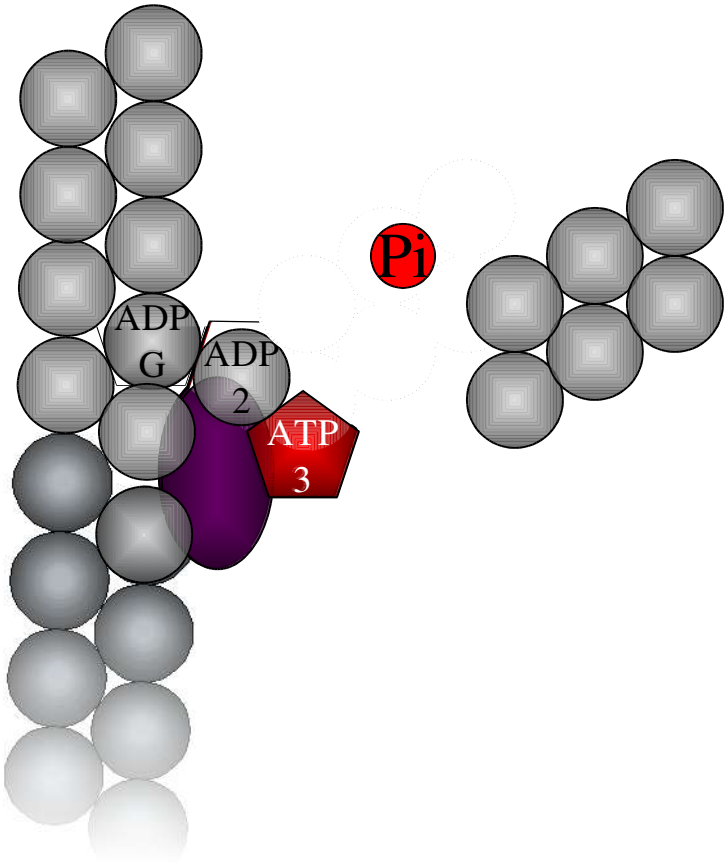
Chasing Leucocyte

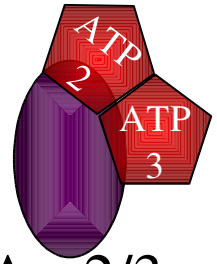
How ATP hydrolysis regulates motility and the stability/mechanical strength of branched actin arrays



Le Clainche et al., J. Biol. Chem. Accel. Publ. 2001; PNAS 2003

TP hydrolysis on Arp2/3 drives filament debranching





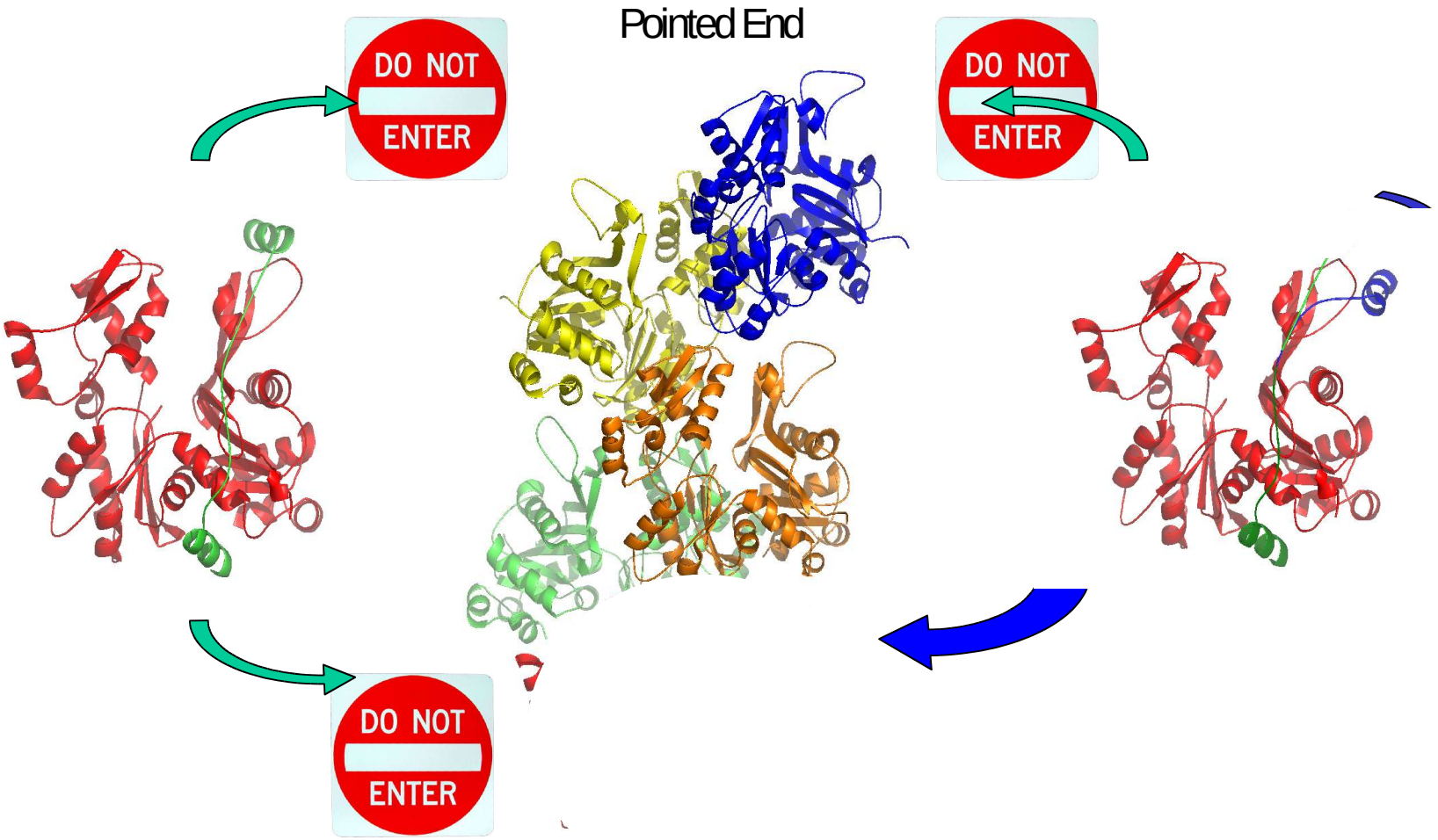
Arp2/3
Inactif

Control of actin dynamics in cell motility

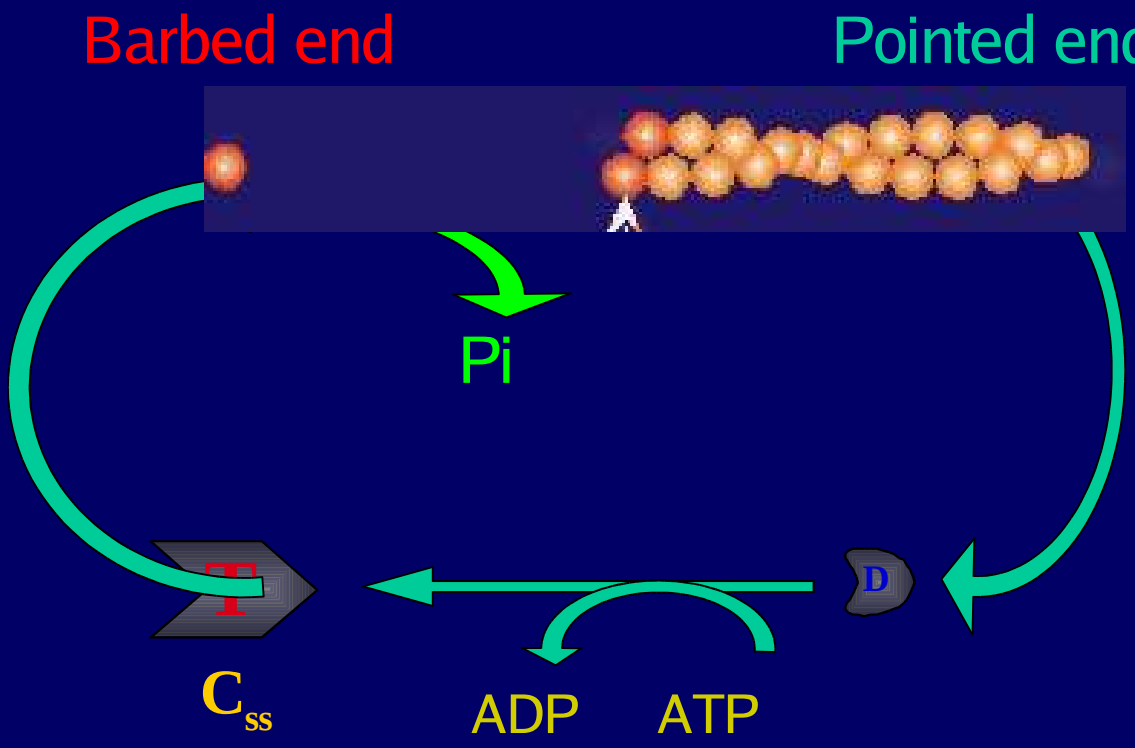
- Control of the [G-actin]/[F-actin] ratio
- Control of filament turnover
- Spatial control of the generation of new filaments (link to signaling)

Structural basis for the switch from inhibition to promotion of actin assembly in ciboulot

(Hertzog et al., Cell 2004)



Treadmilling (Wegner, 1976)

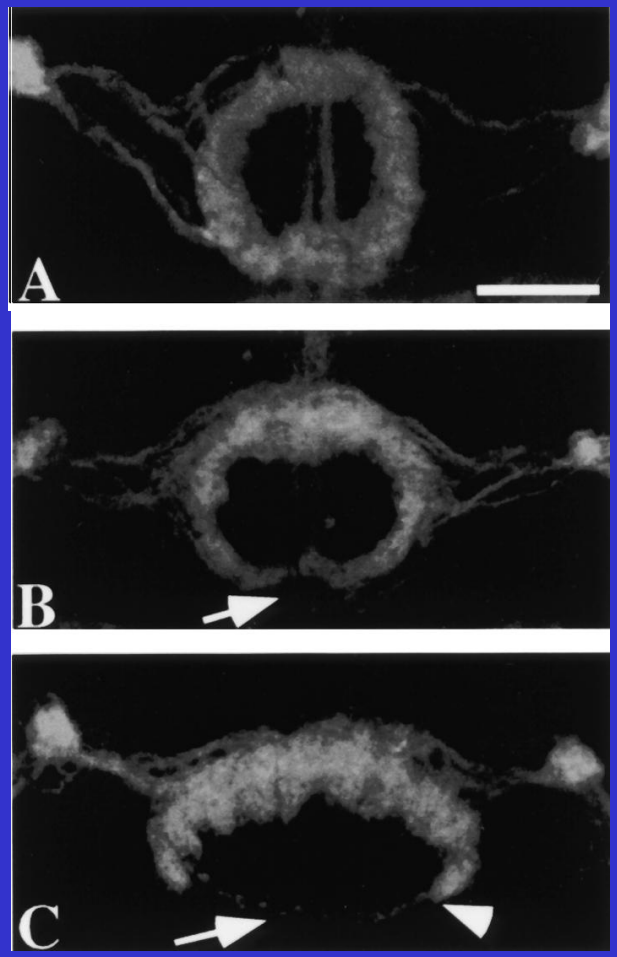


	C_{ss}	Filament turnover
Pure actin:	0.1 µM	3µm / 90 min
Lamellipodium:	2 µM	3 µm / 1 min

Ciboulot regulates axonal growth in *Drosophila* central brain during metamorphosis by acting like profilin

(Boquet et al. Cell, 2000)

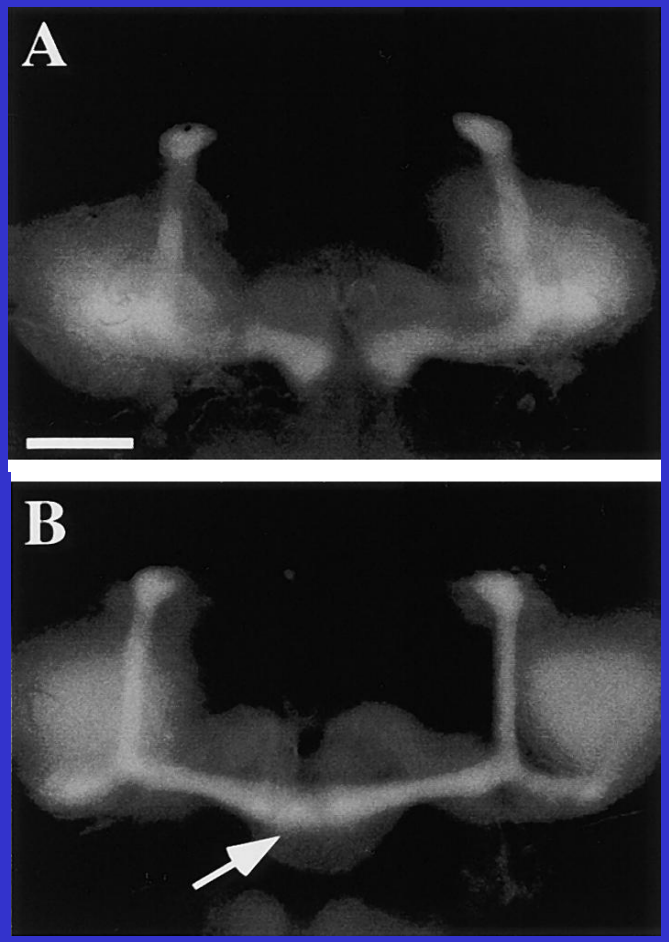
Low level of Cib expression



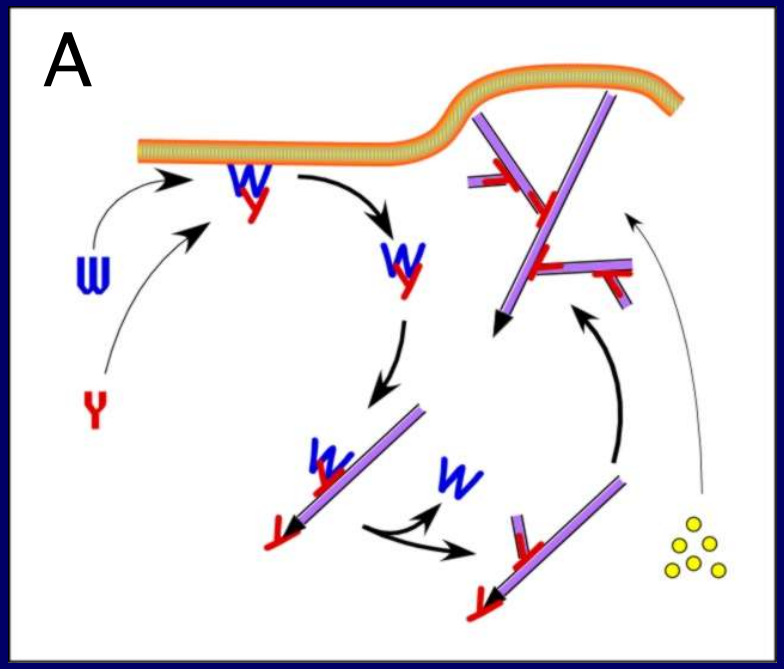
A:wild type

B: mutant

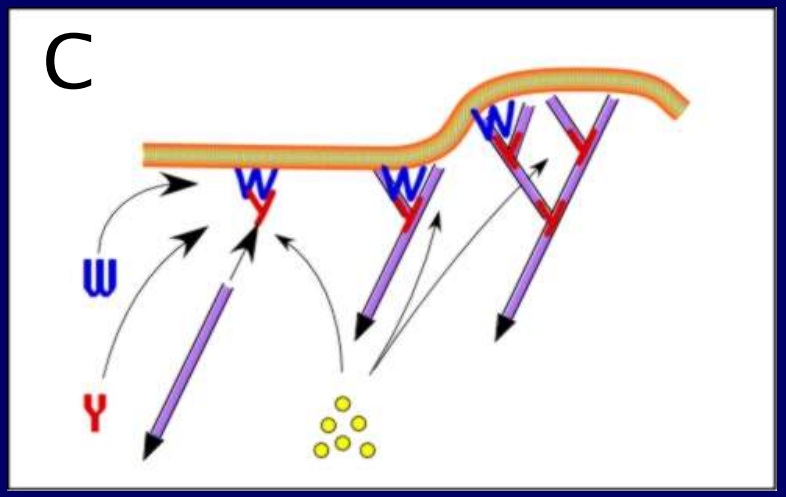
Overexpression of Cib



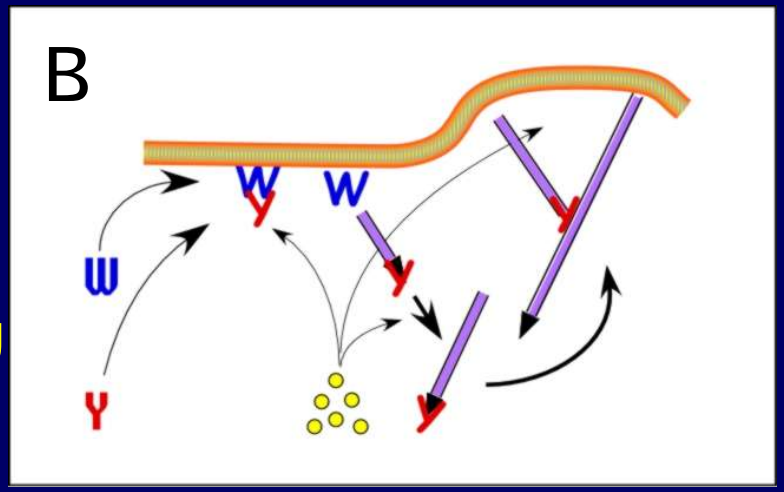
Branching Models



Arp2/3 activation and side branching

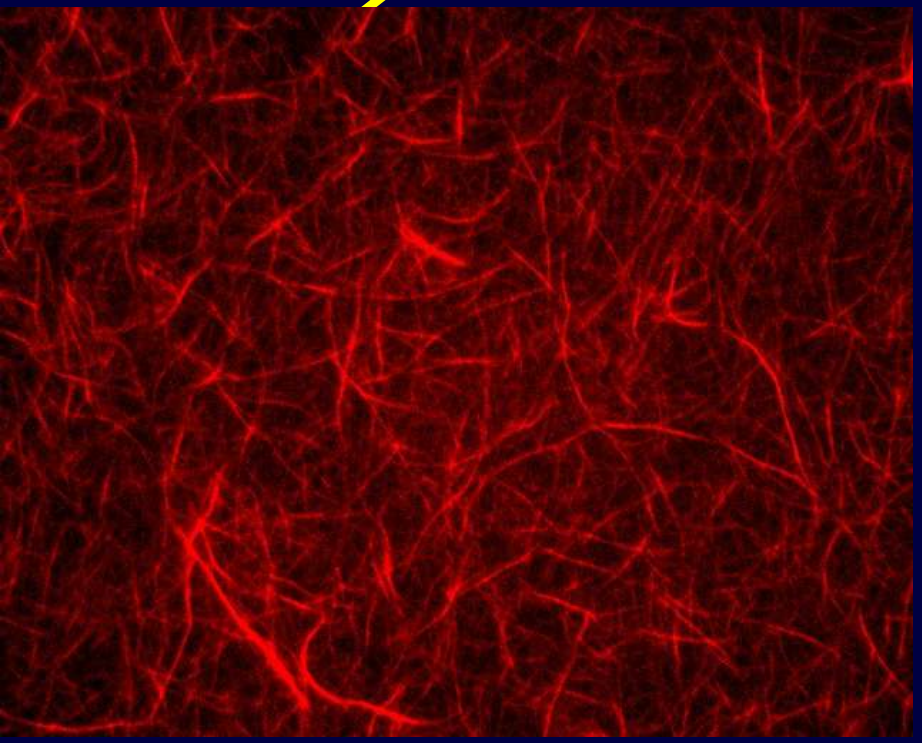


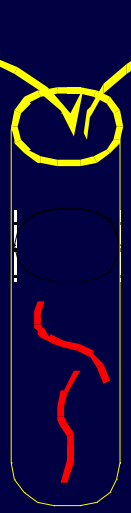
Autocatalytic barbed end branching

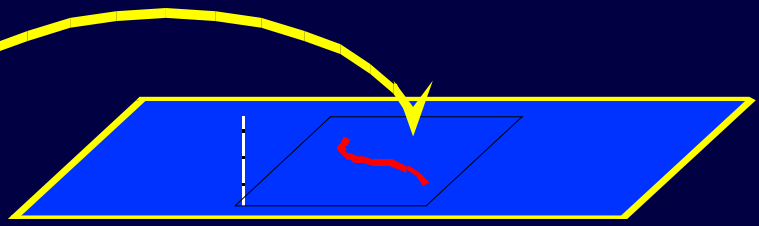


Nucleation at the membrane and side branching

Fluorescence Video Microscopy of F-actin

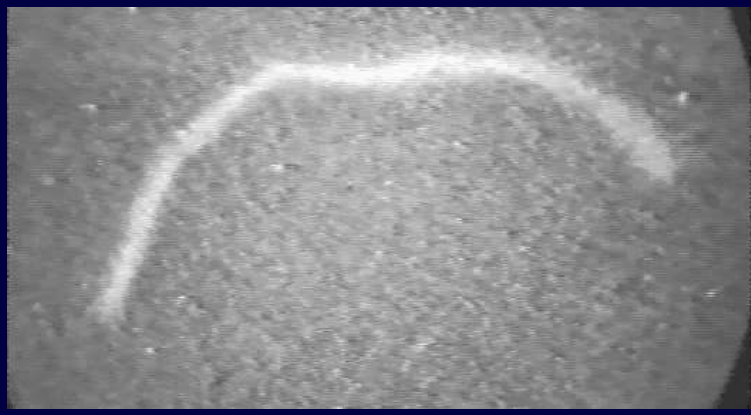






1 filament per field
= 1 filament / 50 picoliter

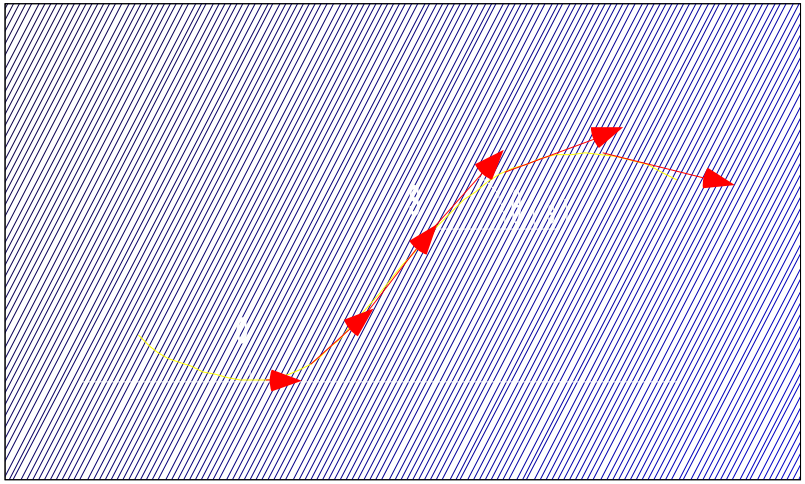
F-Rh-actin
20 nM
+ G-actin
=Cc



Isambert, Venier, Maggs and Carlier (1995)

Flexibility of Actin Filaments

Correlation of tangential directions

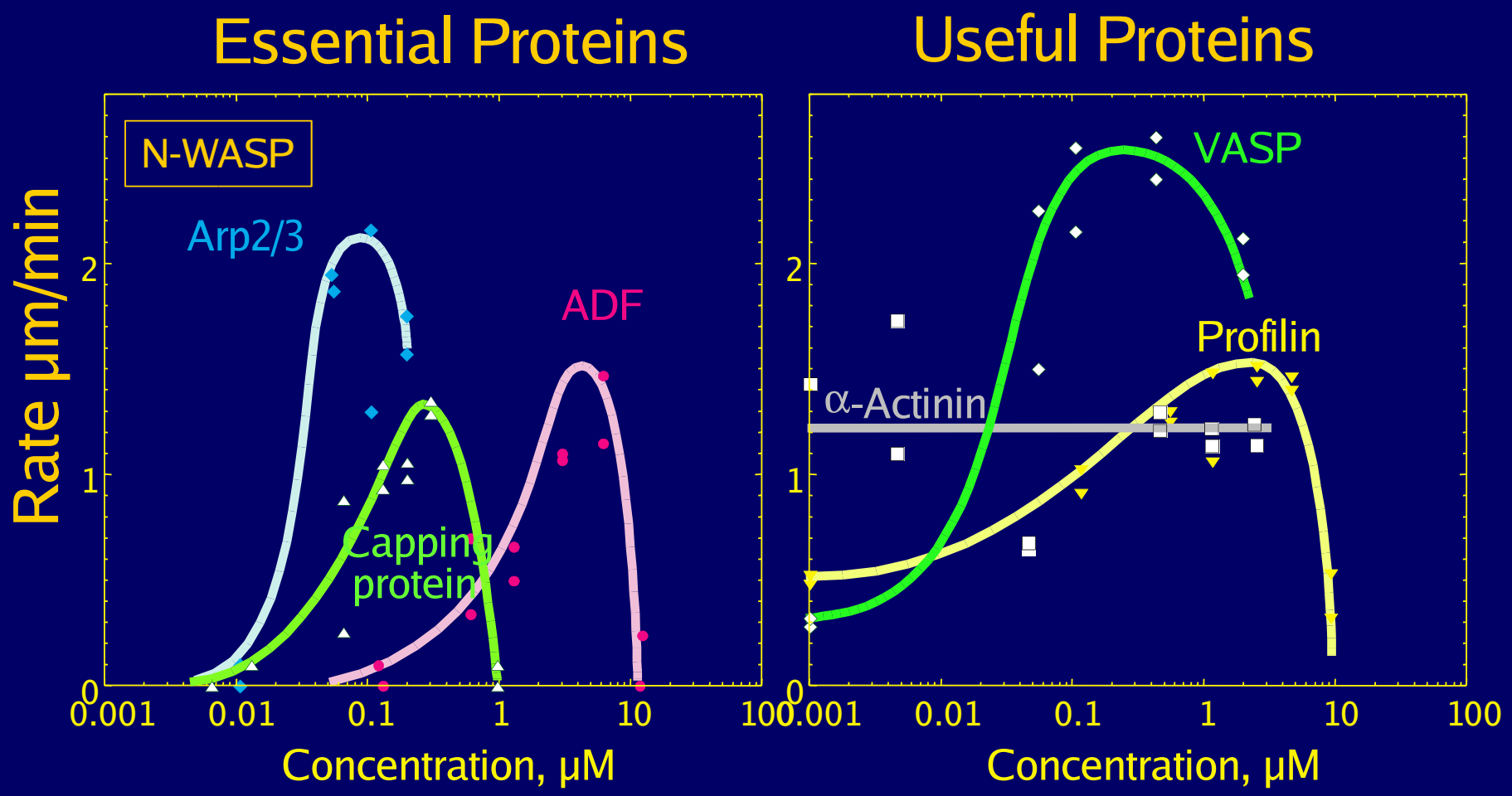


$$\langle c(s) \rangle = \cos[\theta(s) - \theta(0)]$$

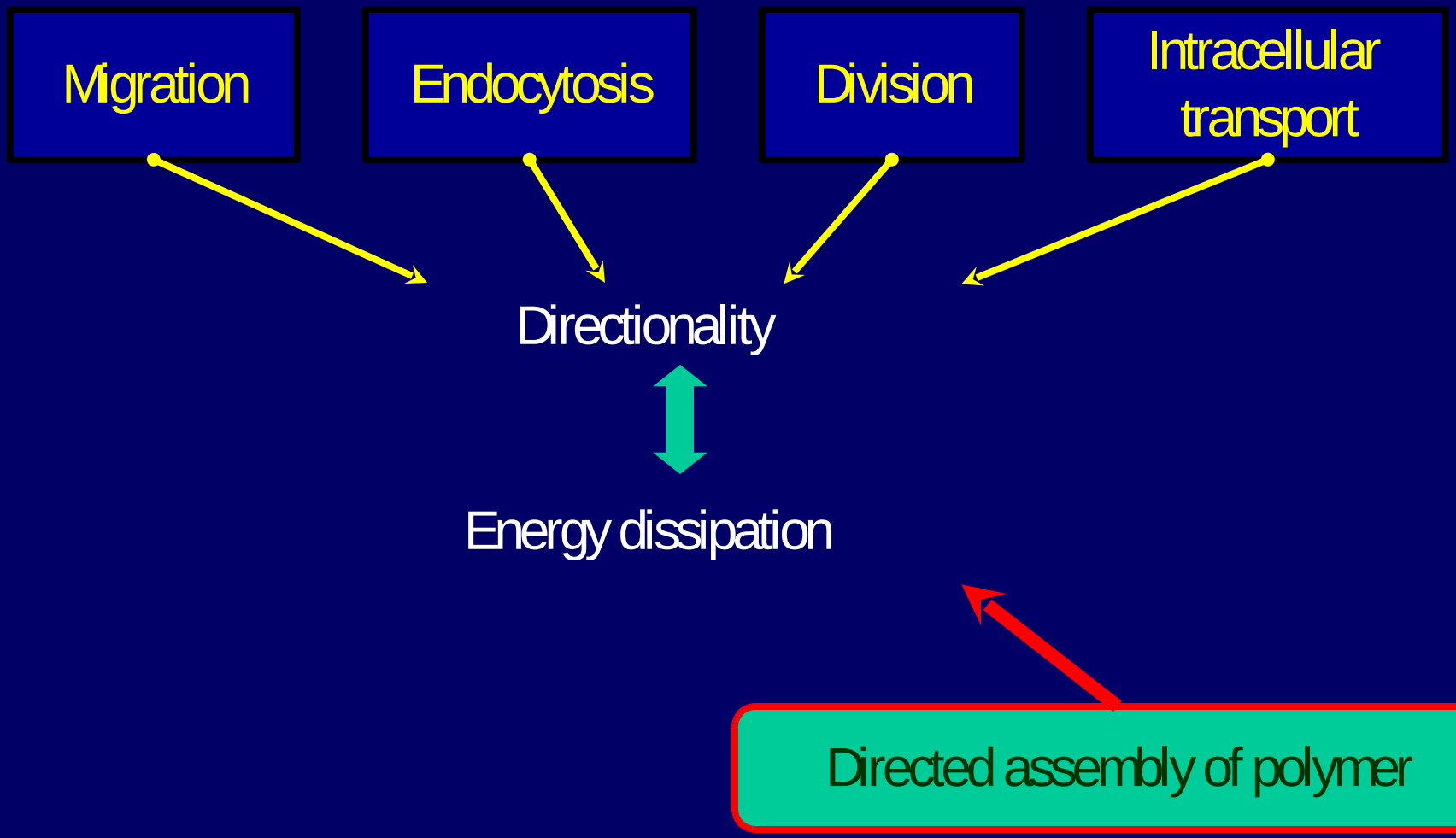
$$\langle c(s) \rangle = e^{-|s|/2L_p}$$

$$\ln \langle c(s) \rangle = -\frac{s}{2L_p}$$

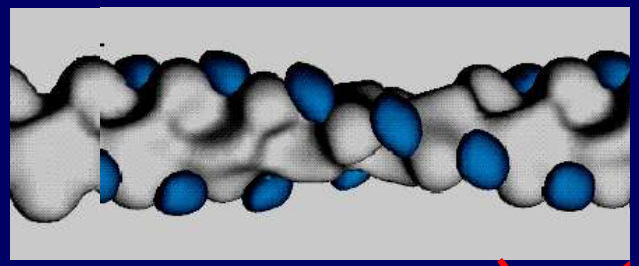
Reconstitution of actin-based movement from pure proteins

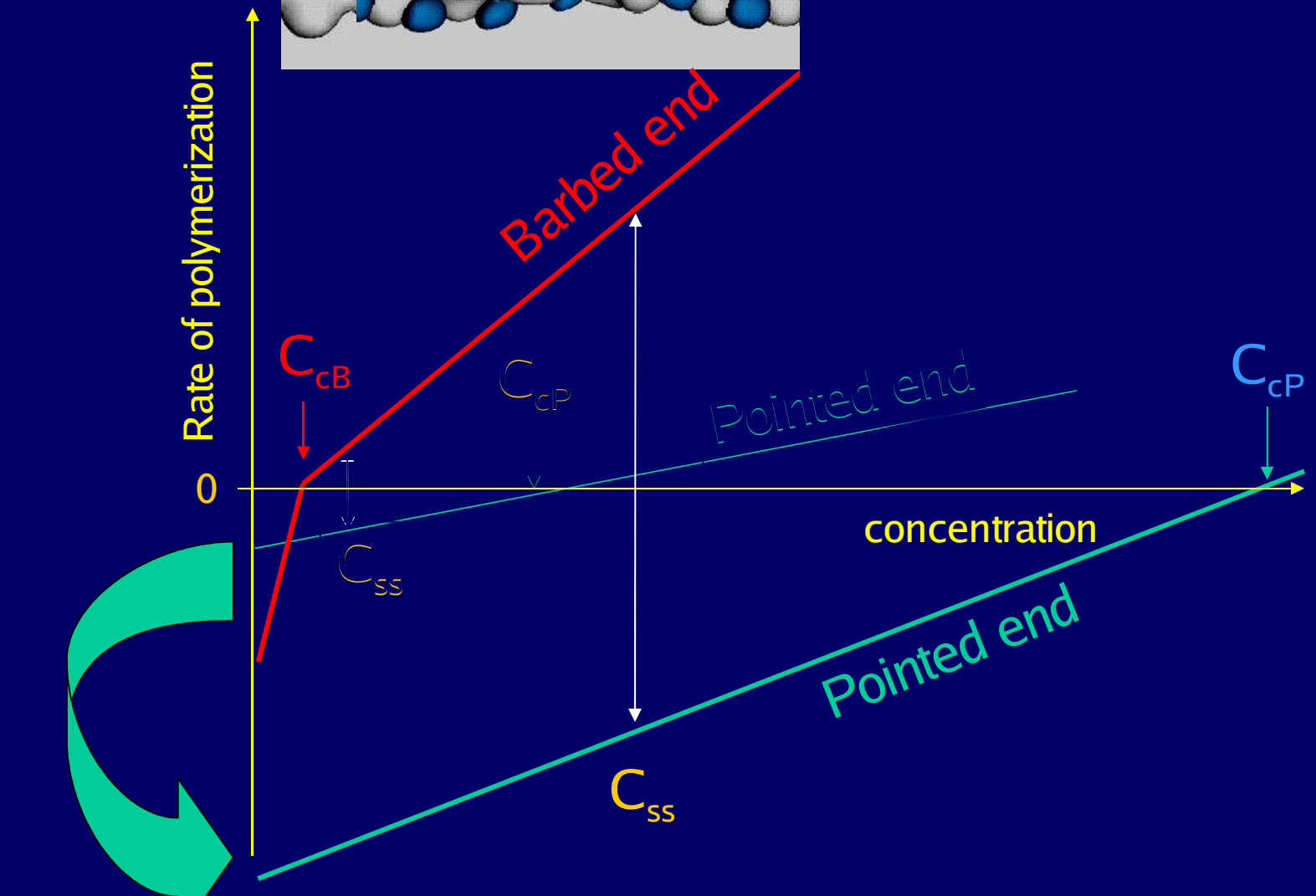


Motile activities of living cells



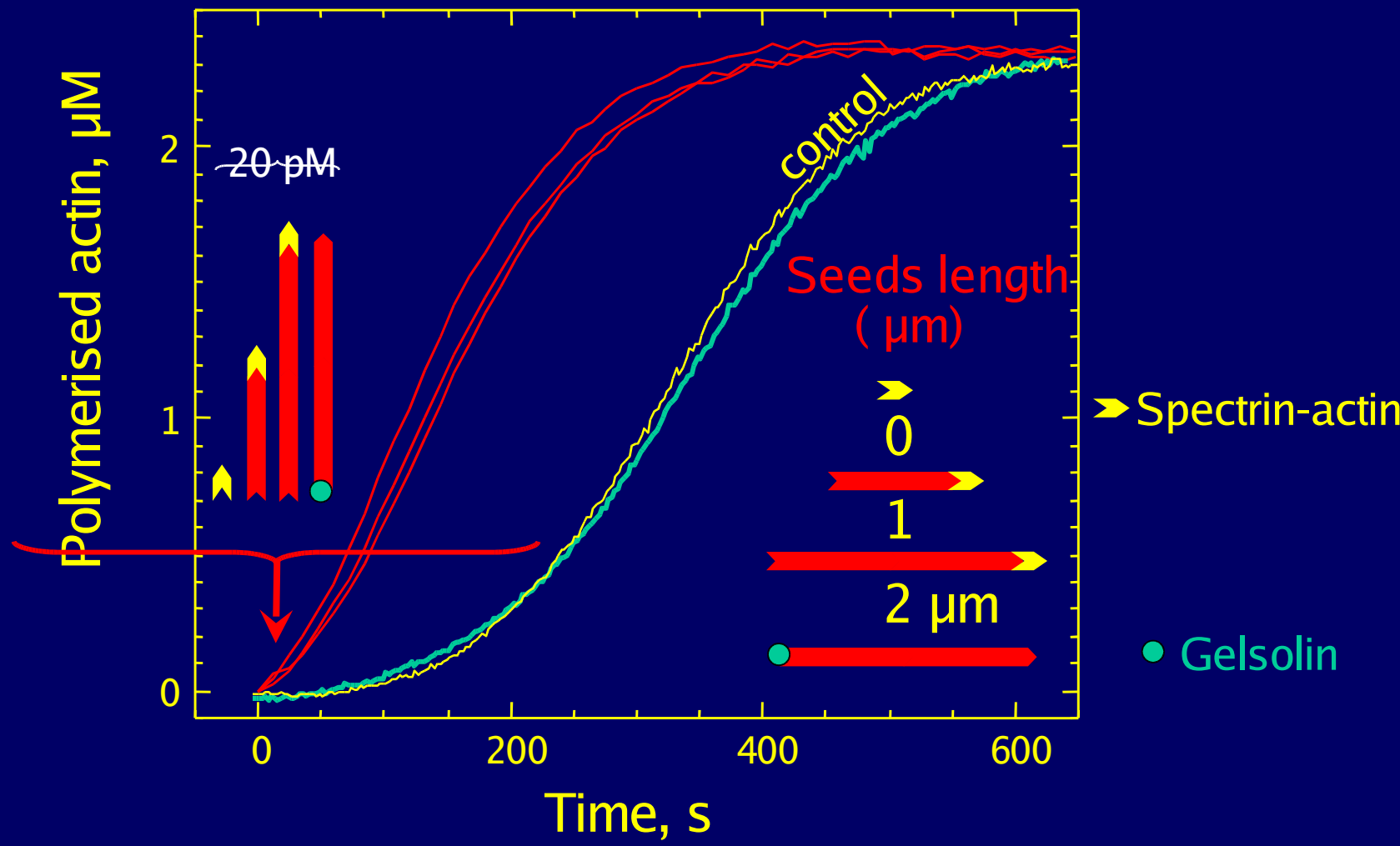
ADF increases the treadmilling of F-actin





Arp2/3 interacts with barbed ends, independently of filament length

(D.Pantaloni *et al.* 2000)



VASP increases the rate of detachment of the branched junction from ActA, allowing growth after branching