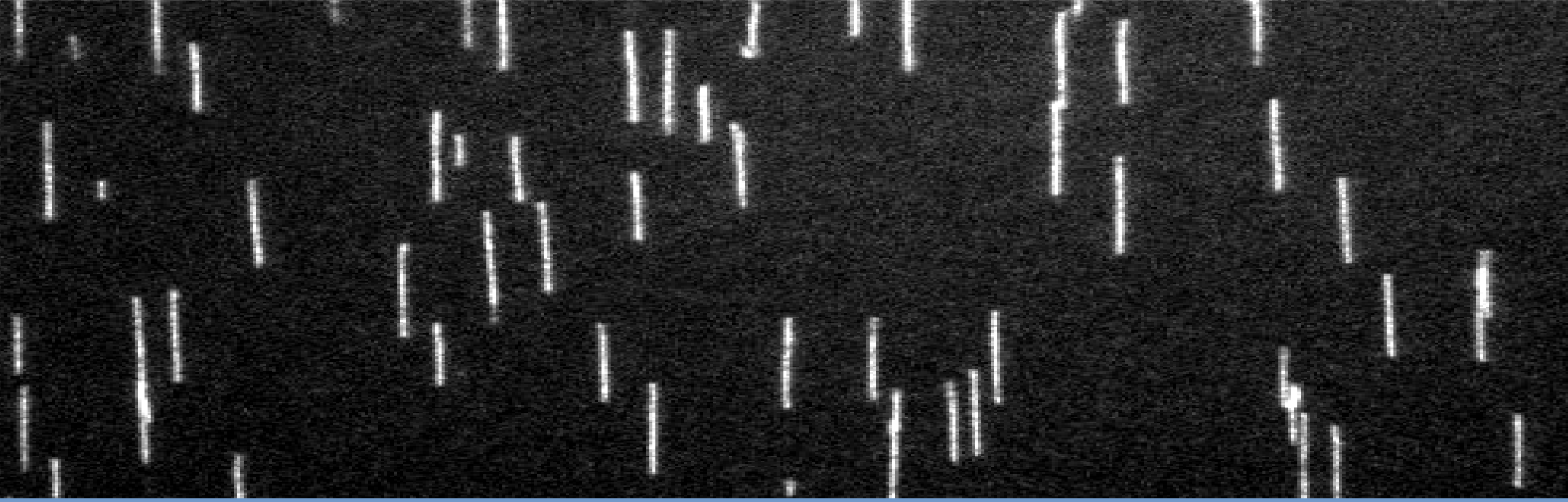
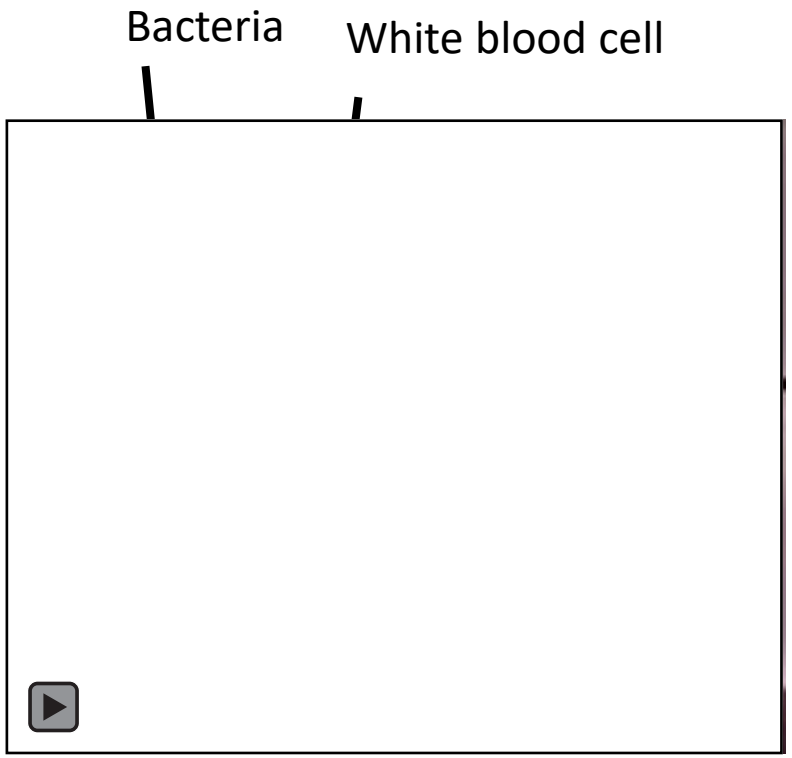


Drunken sailors and sober cells, the ways they move

Shashank Shekhar

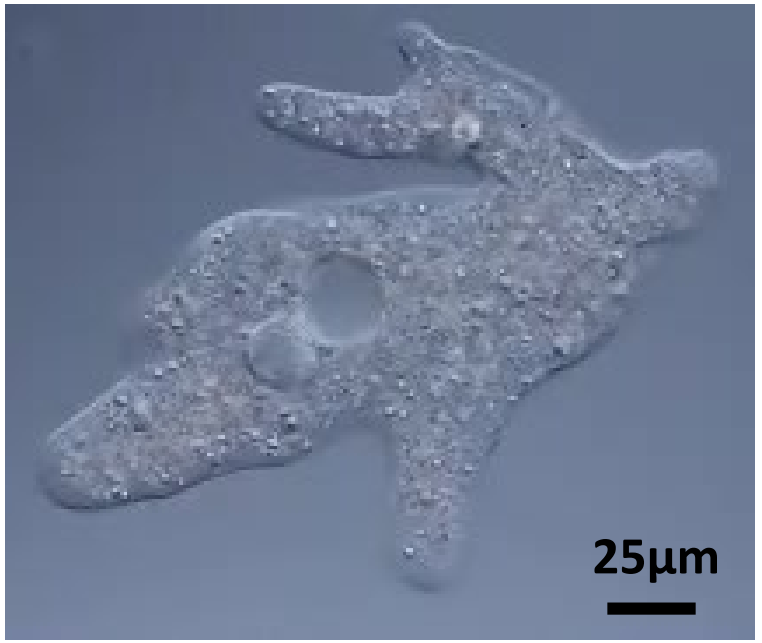
Brandeis University

Cell motility is conserved across billions of years of evolution



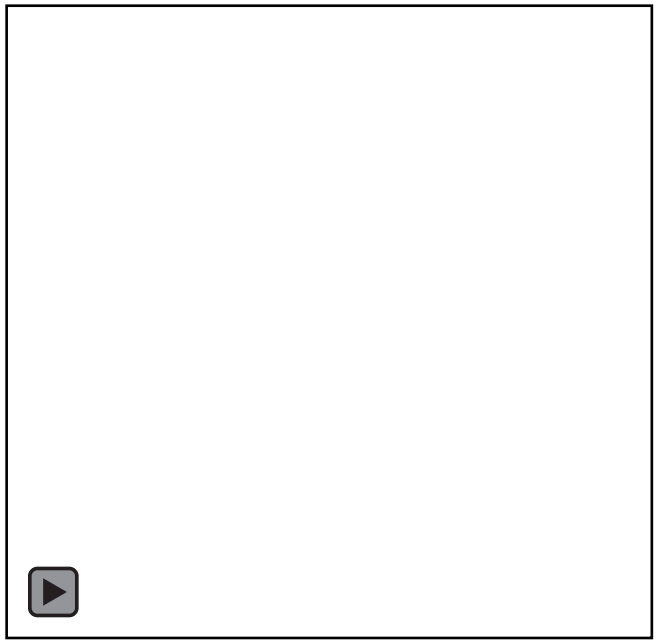
White blood cells
(Slightly speeded up)
David Rogers, 1950
Blood smear

100,000 years ago



Amoeba
(Real time)
Feeds on bacteria in soil

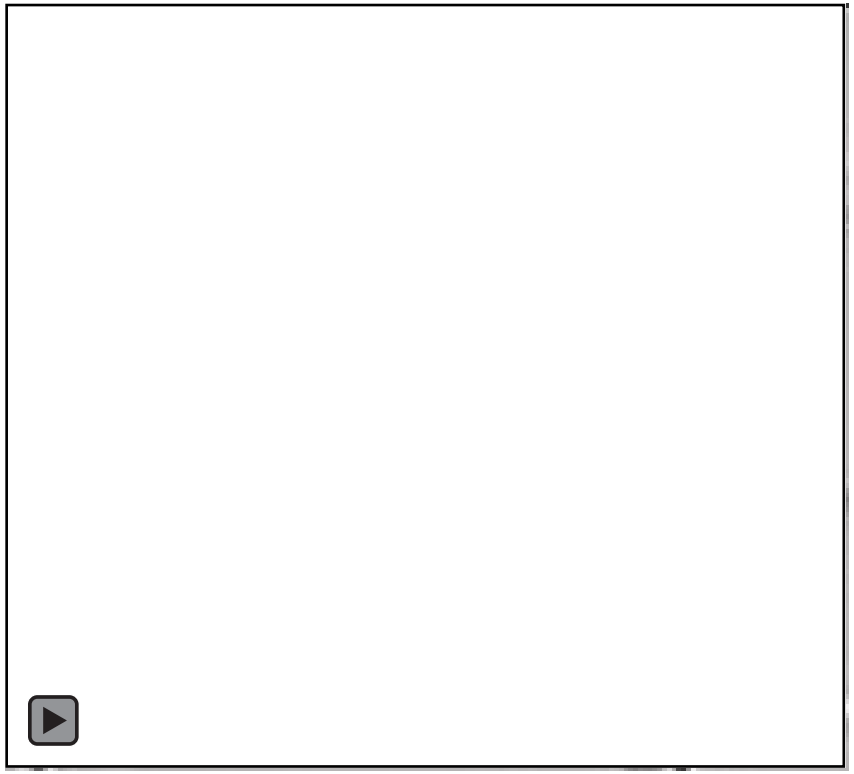
2 billion years ago



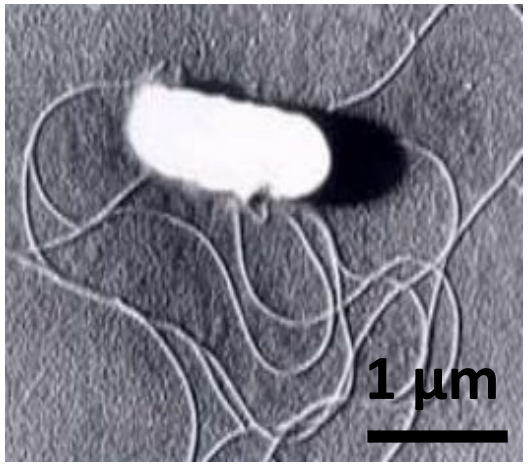
Fish scale cell
(30 X speeded up)
Theriot lab
Wound healing

500 million years ago

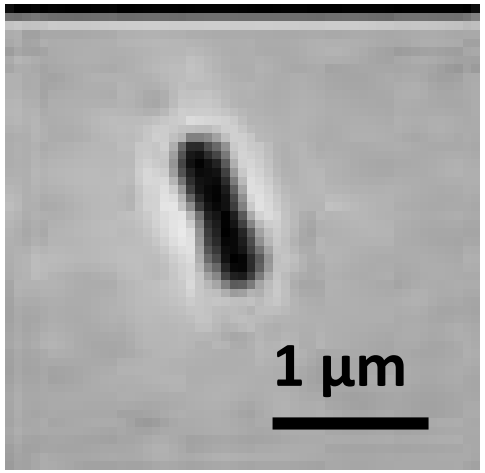
"Run and Tumble" motility of Listeria Monocytogenes



Listeria's "Run and Tumble"



< 30°C



~ 37°C

Gründling et al, 2004

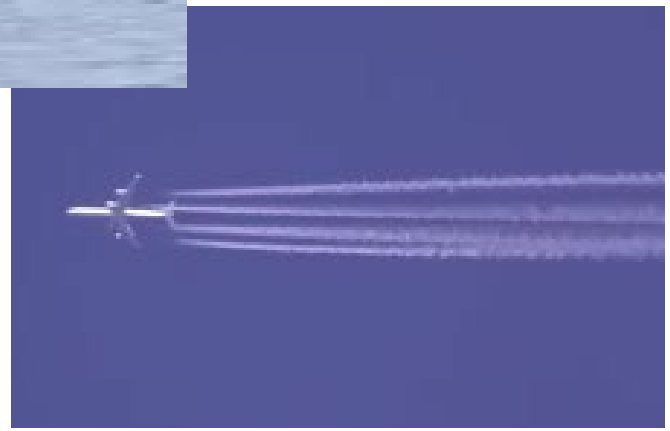
Intracellular pathogens also show similiar behaviour

Listeria Bacteria
Theriot lab (60X speeded up)

Comet tail

Speedboat

Contrails

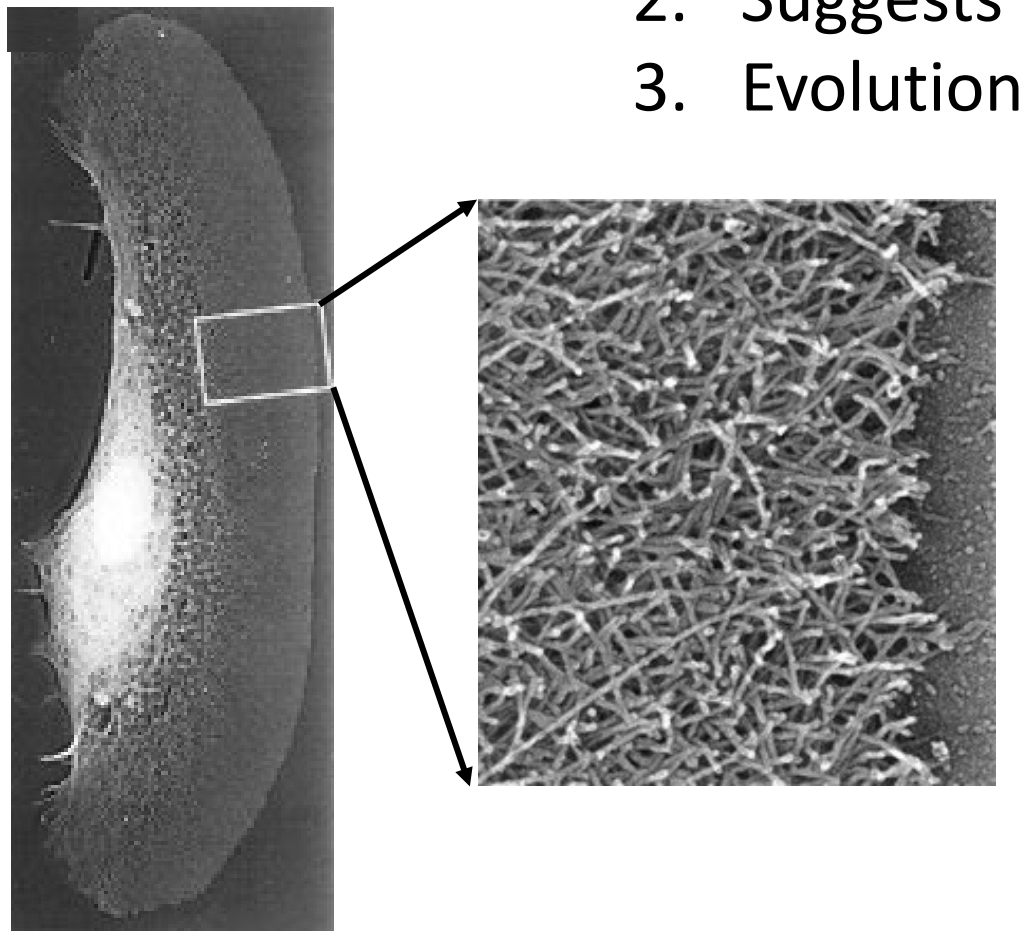


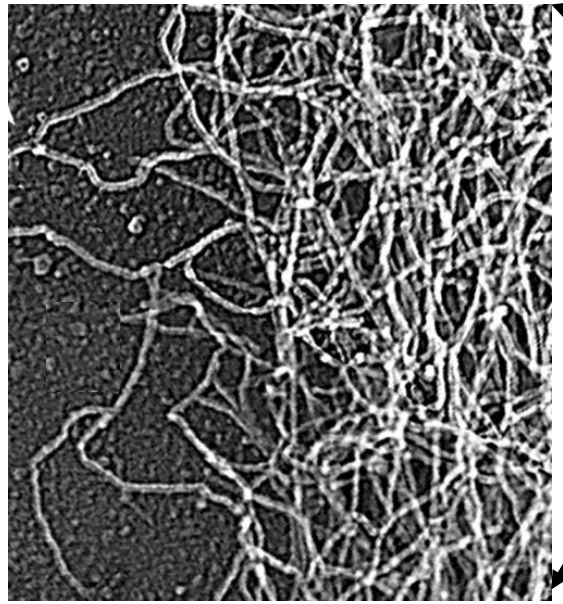
Cellular and pathogenic motility have the same molecular basis

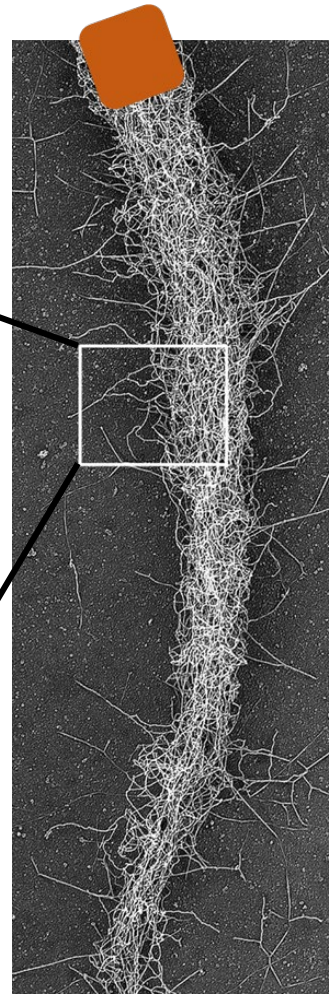
Fish scale cell

1. Similar rates of propulsion (~µm/min)
2. Suggests identical molecular basis.
3. Evolutionary conserved

Pathogen



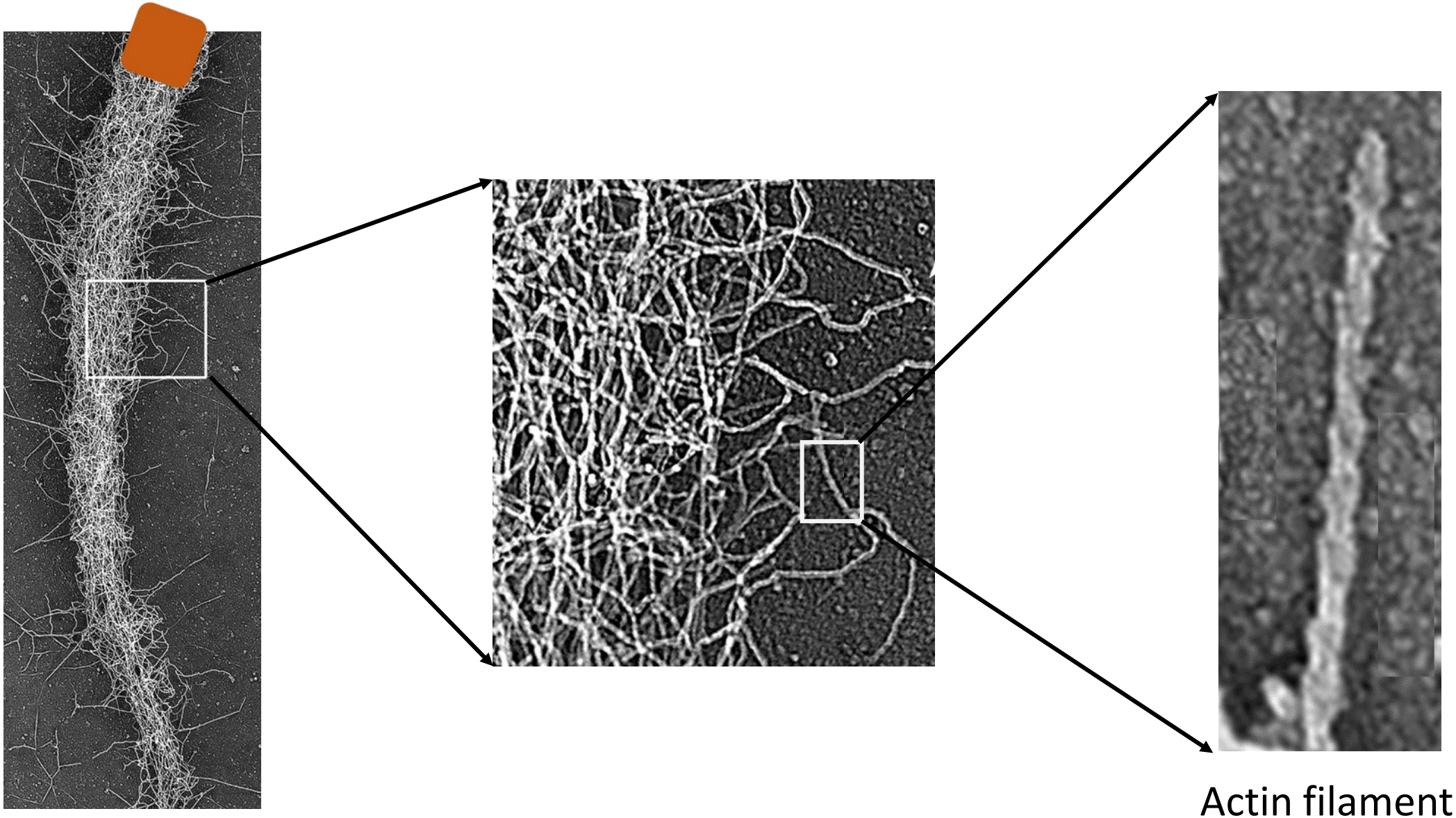




Svitkina et al 1997

Cameron et al, 2001

Bacterial and cellular motility have the same molecular basis

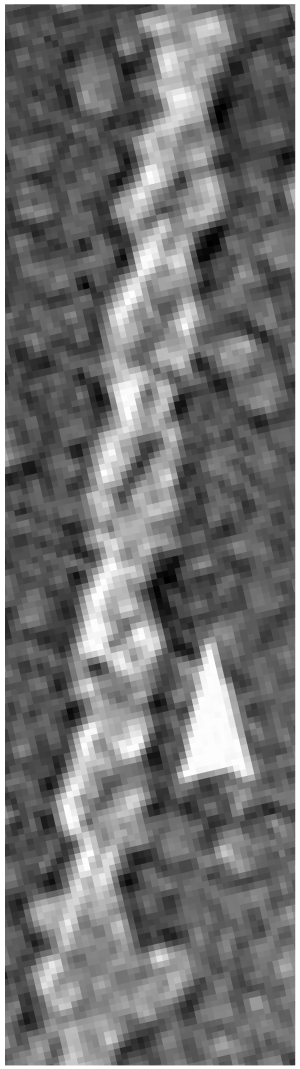


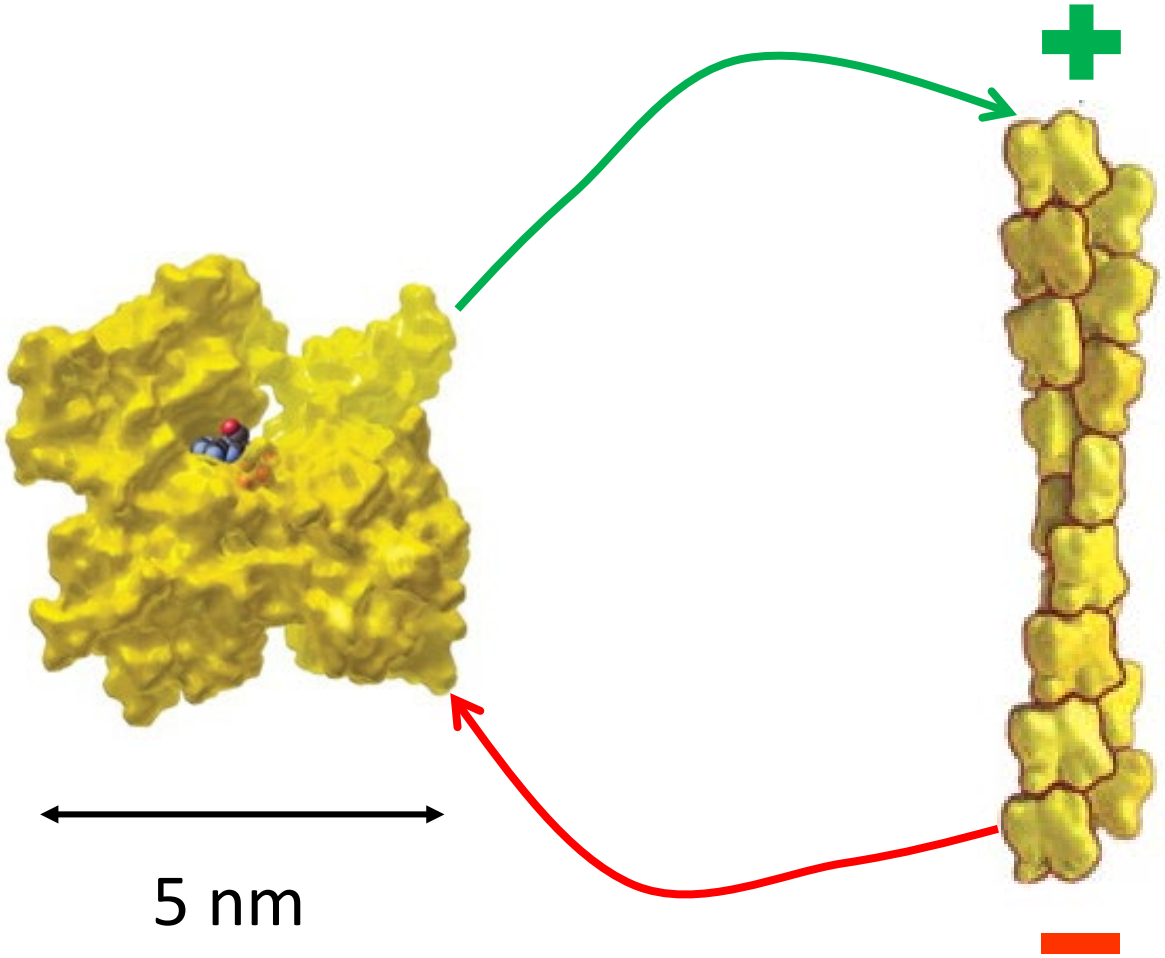
Actin filament

Svitkina et al 1997; Gary Borisy

Cameron et al, 2001

Actin filaments are formed from monomers



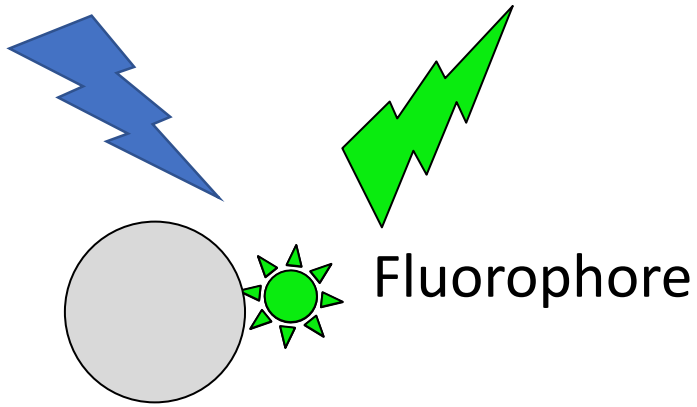


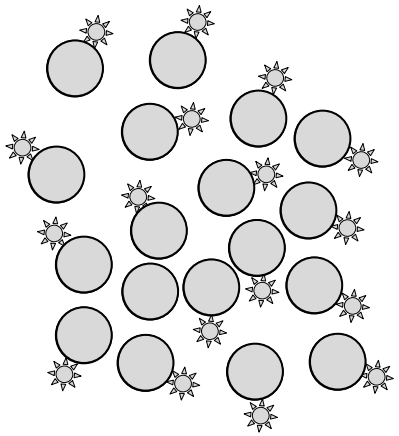
How do such small molecules self-assemble to form structures orders of magnitude bigger

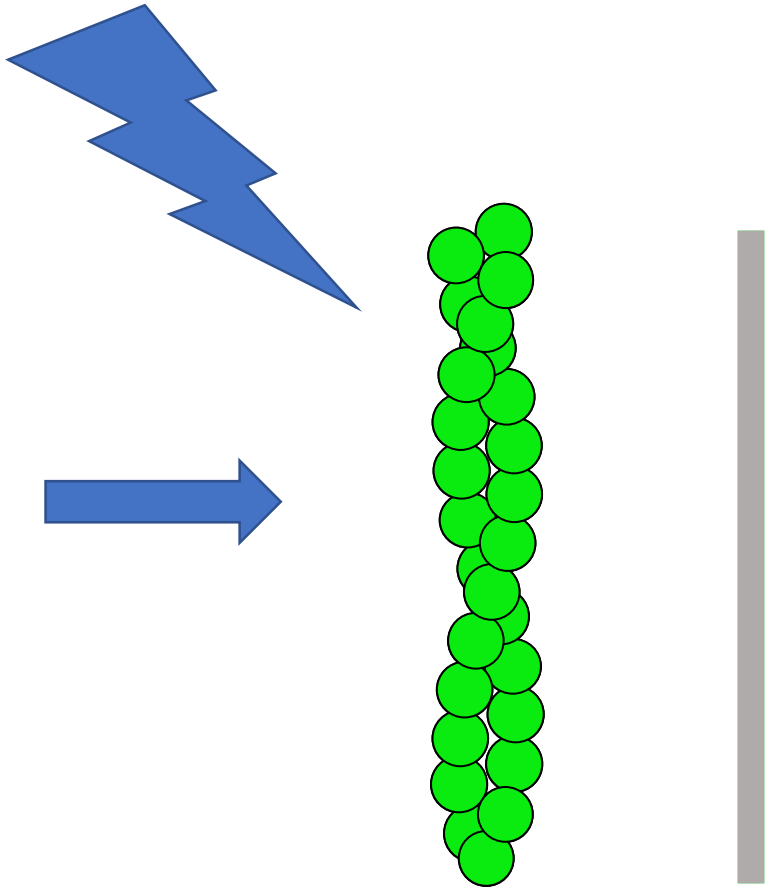
Pollard and Cooper, 2001

Fluorescence microscopy enables direct visualization of actin filaments

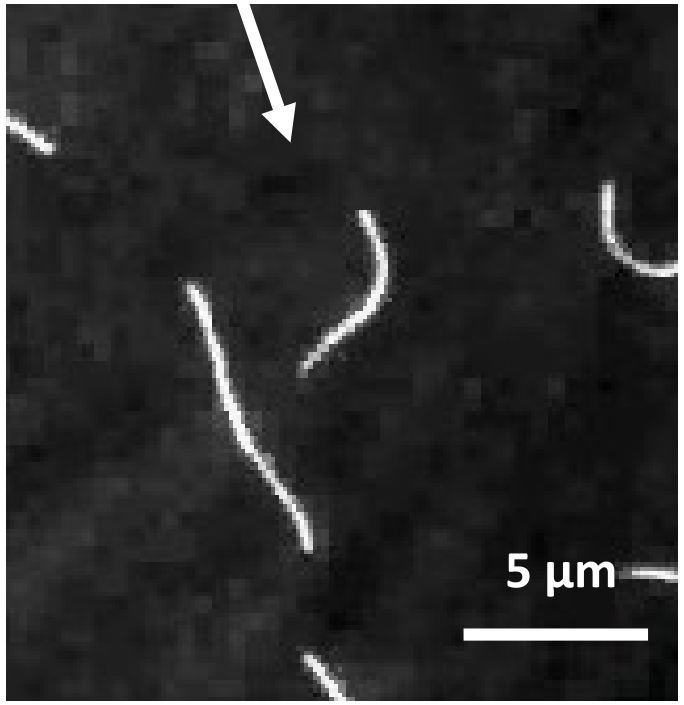
Actin monomer

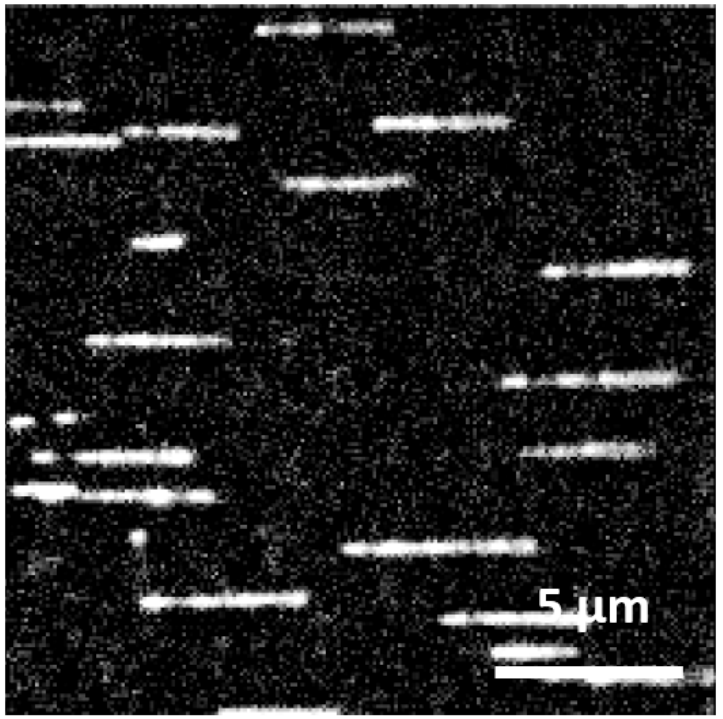




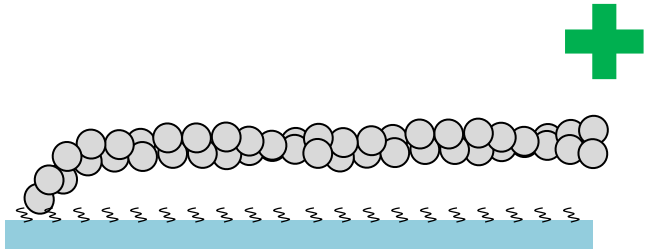


Fluorescence microscopy enables direct visualization of actin filaments





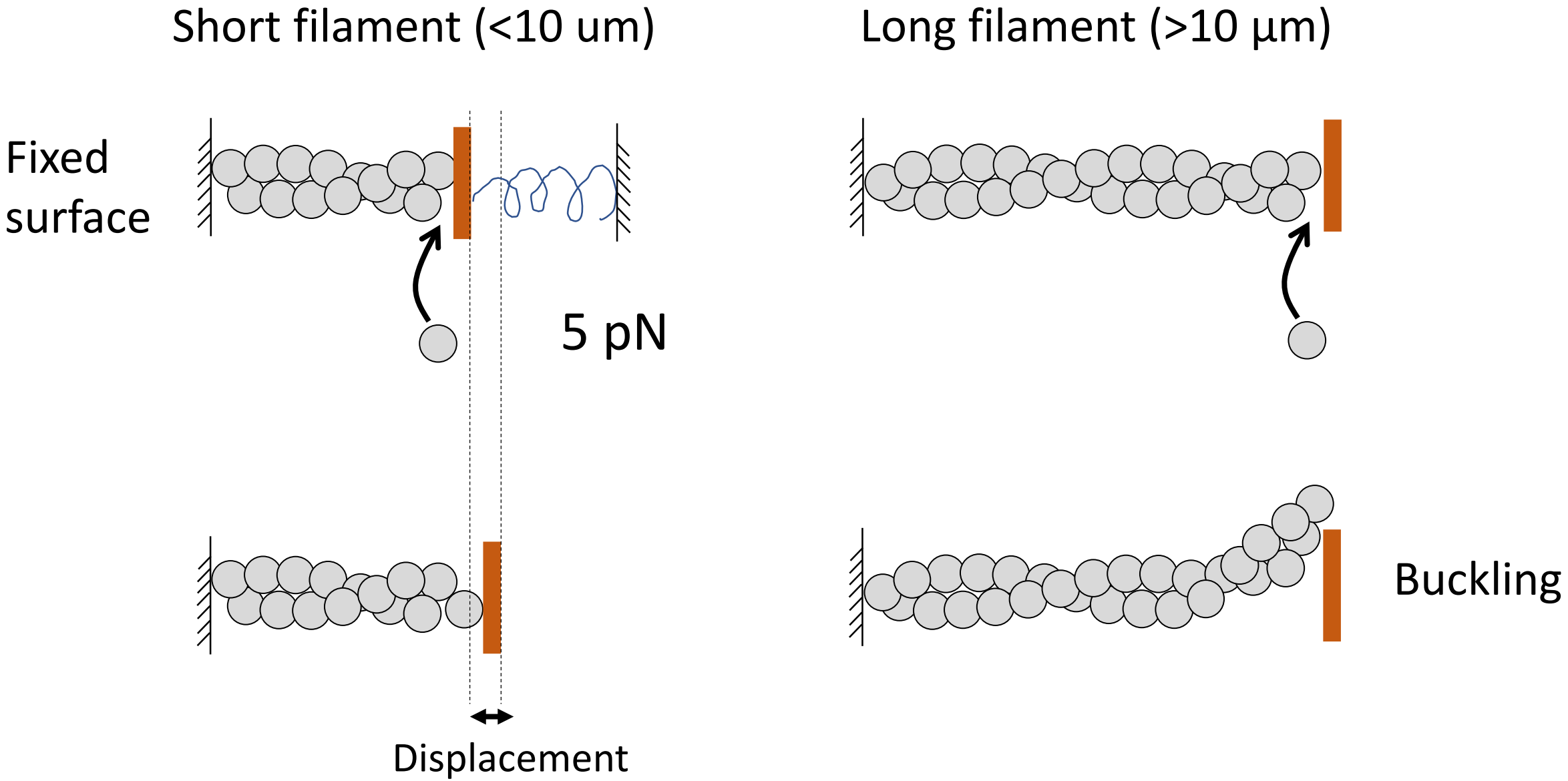
Flow



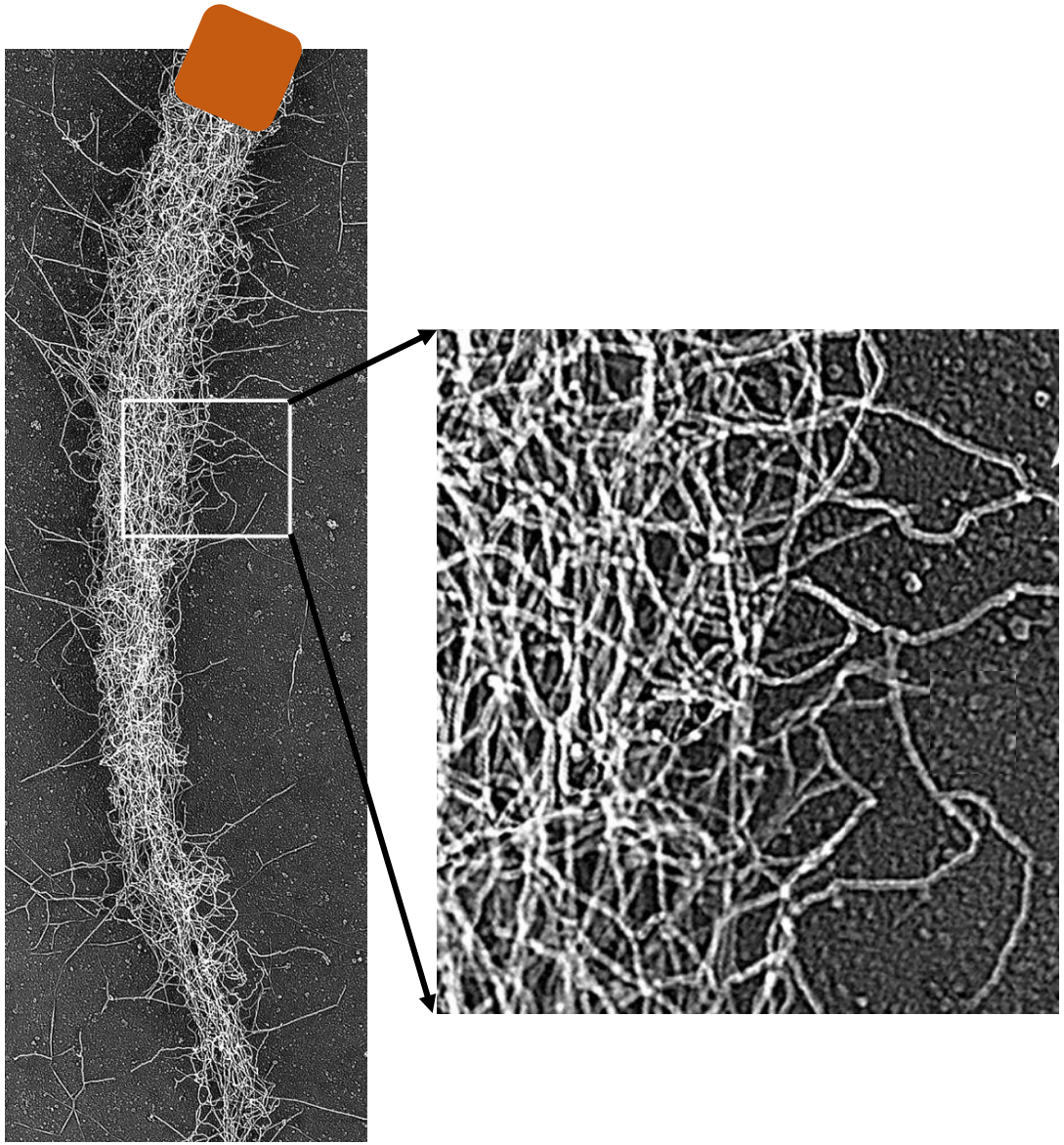
Flow

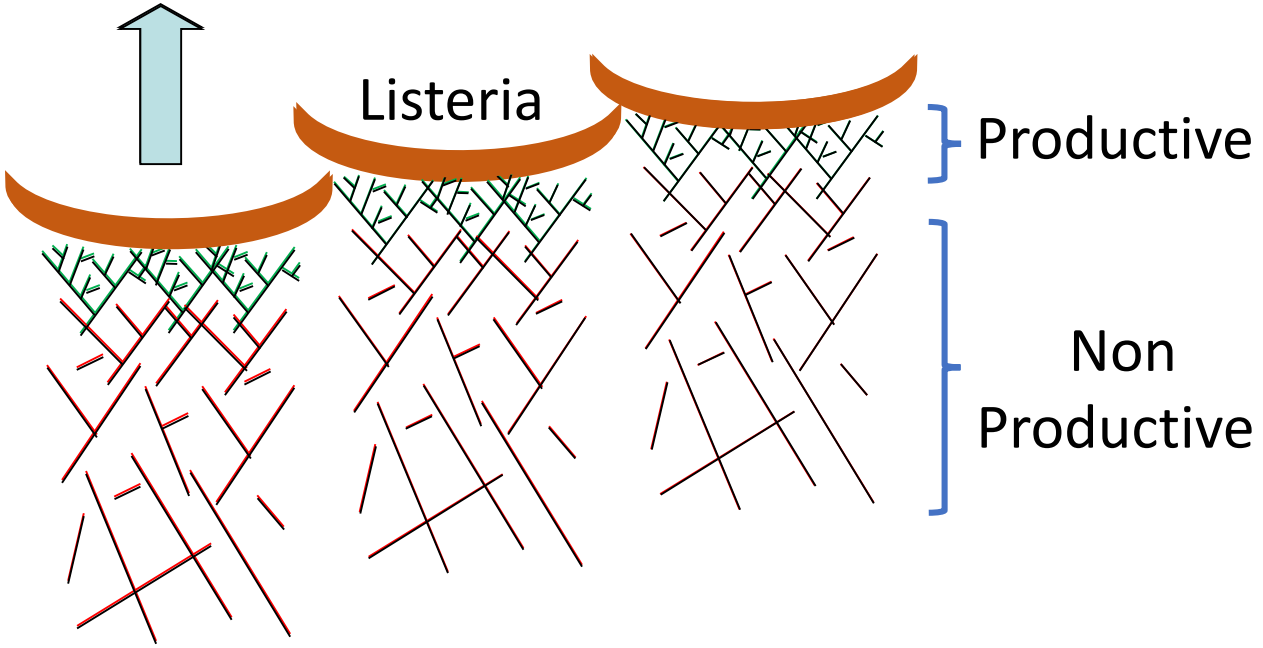
- Filament growth
- Thermal fluctuations

Actin polymerization can generate force



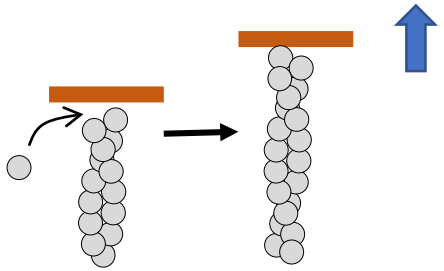
Filament networks enable overcoming buckling



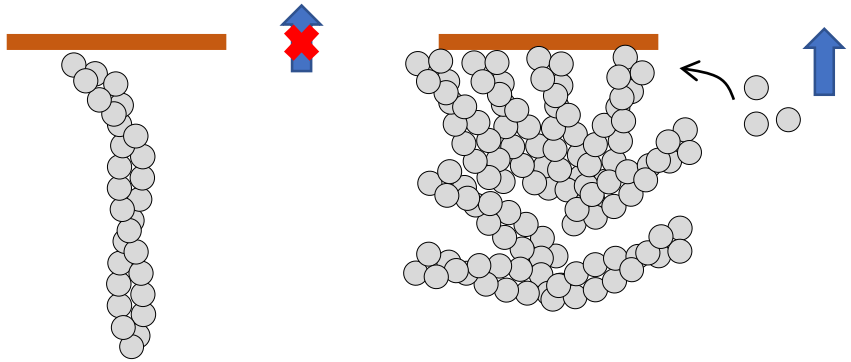


Requirements for actin propulsion

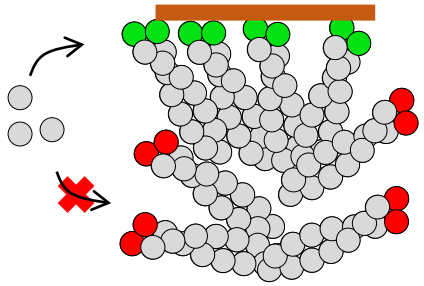
Polymerization = force



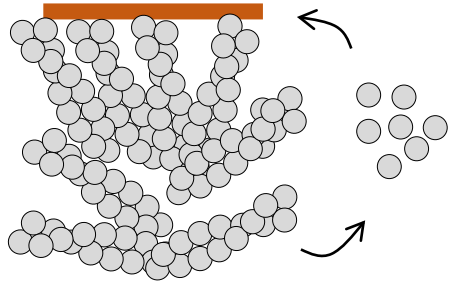
Filament mesh prevents buckling



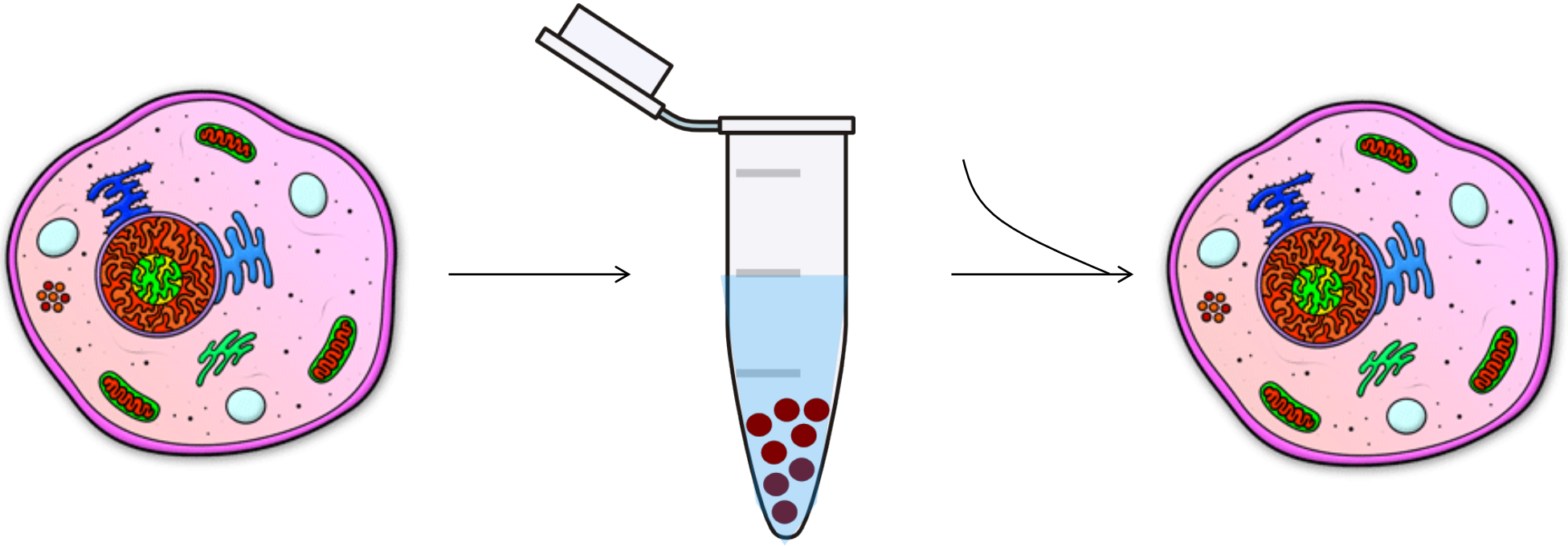
Elongate productive Filaments



Regeneration of monomers



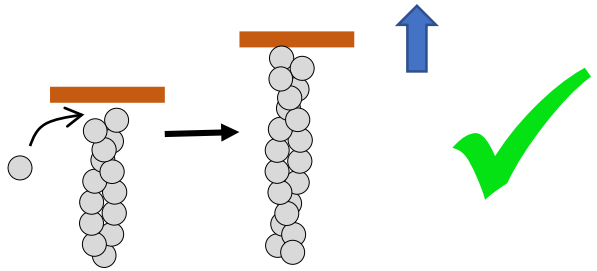
Approach : What I can not create, I don't understand.



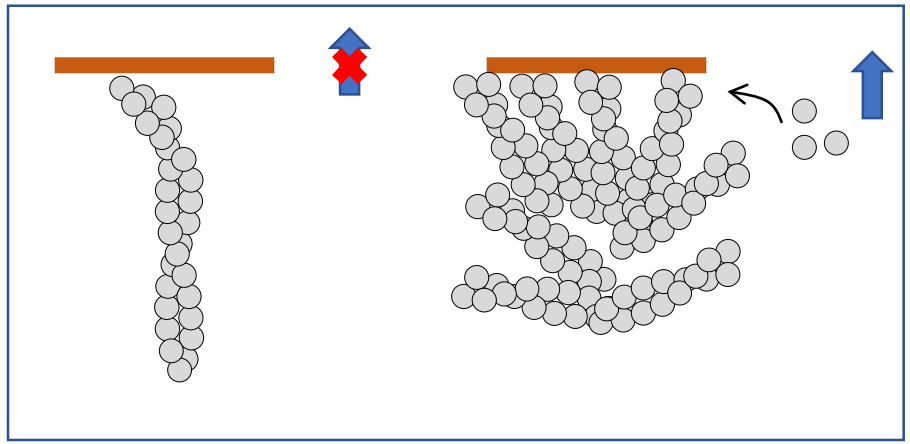
1. Identify essential components
2. Characterize the component's function
3. Reconstitute function i.e. motility

Requirements for actin propulsion

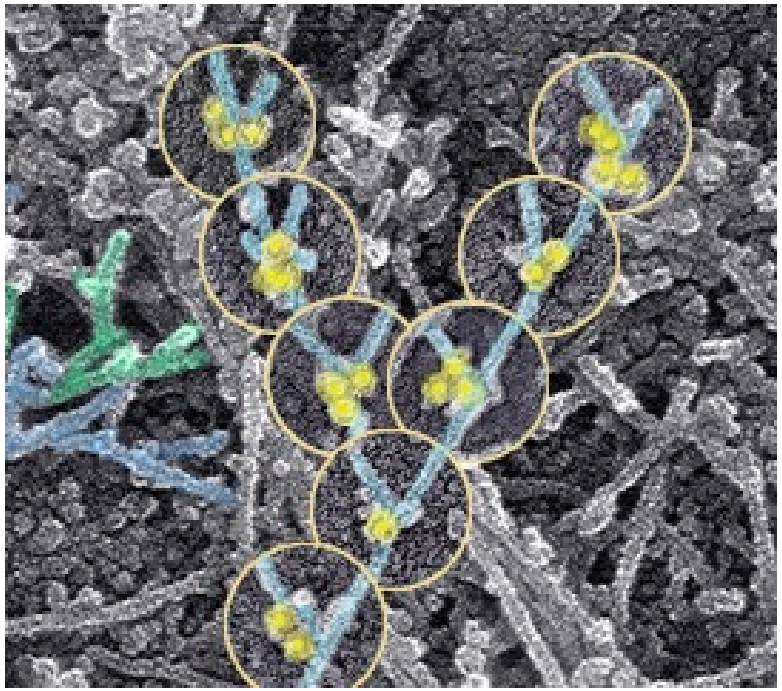
Polymerization = force



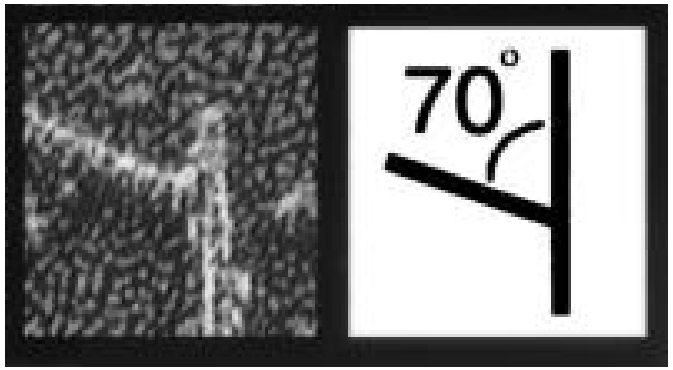
Filament mesh prevents buckling



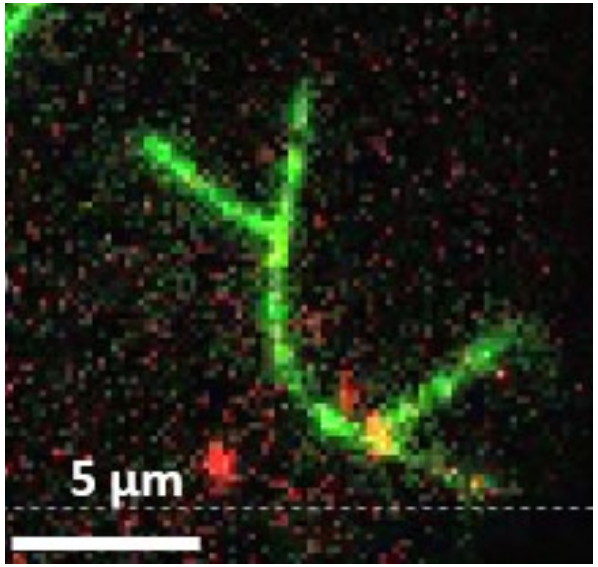
“Branchers” branch actin filaments to form a dense mesh



Svitkina & Borisy, 1999

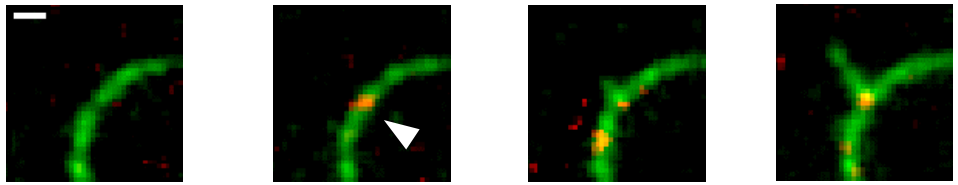


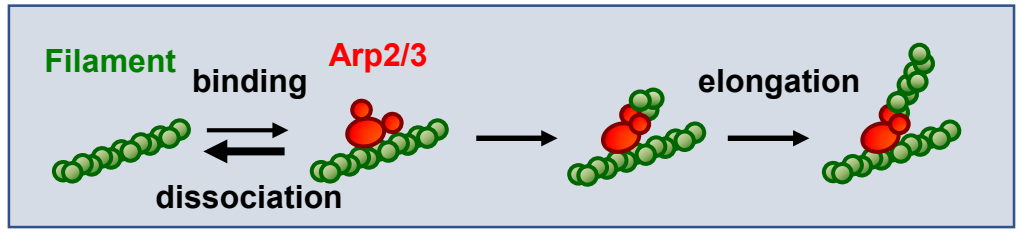
Mullins et al., 1999



Actin

Brancher
(Arp2/3)

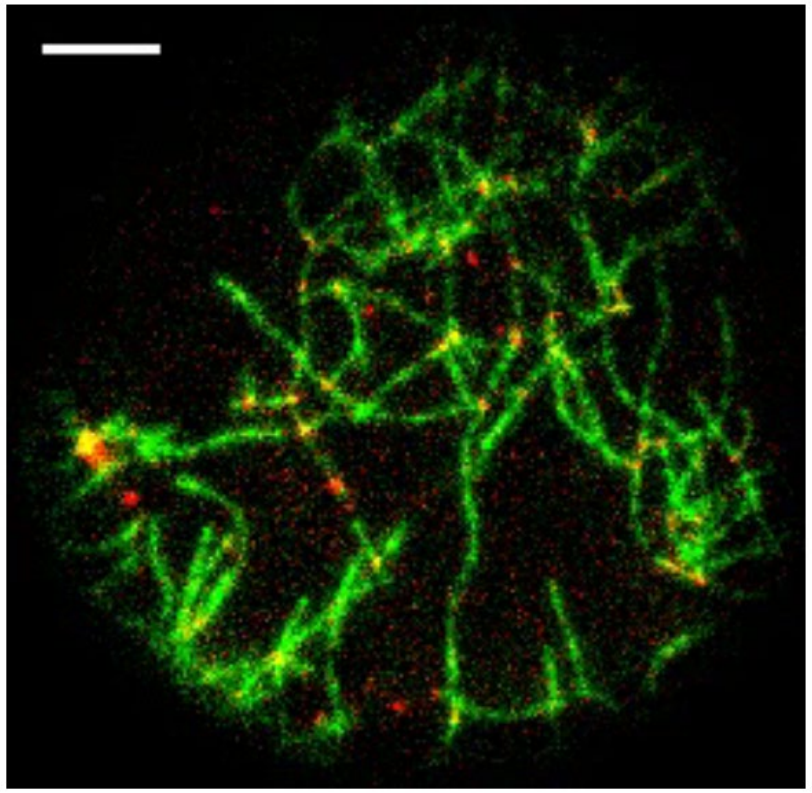




Smith et al, 2013; Gelles and Goode la

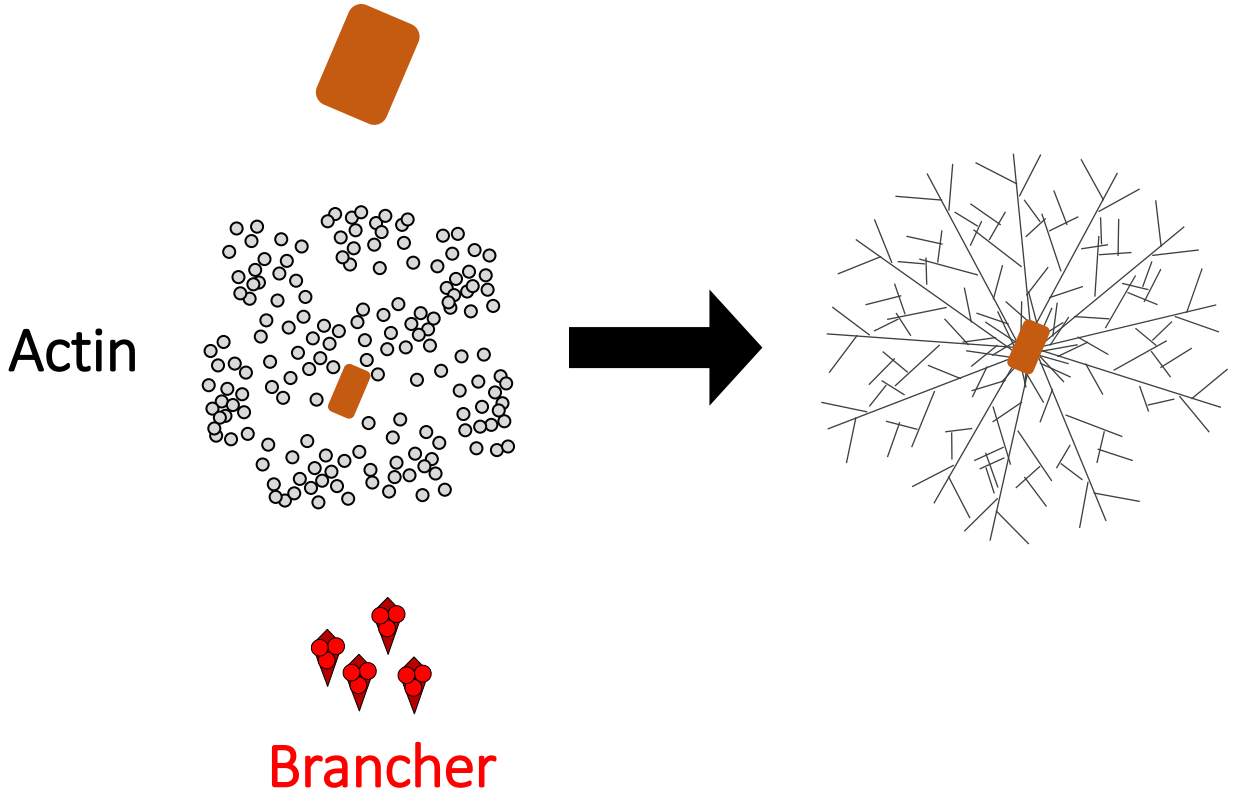
Requirement 1 : Generating a dense mesh of filaments

Dense branching forms a mesh



Actin Brancher

Listeria mimicking bead

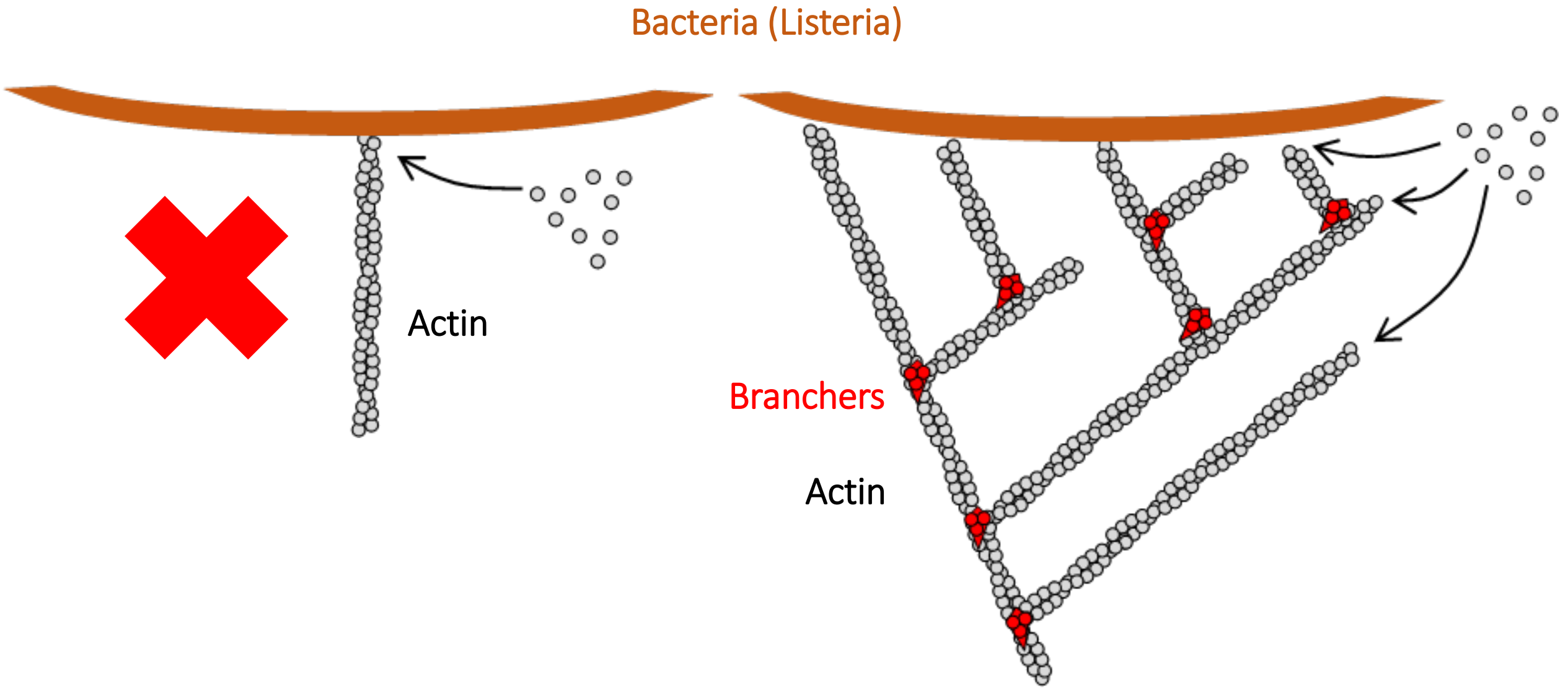


Listeria placed in mix of Actin + Arp

Gel formed

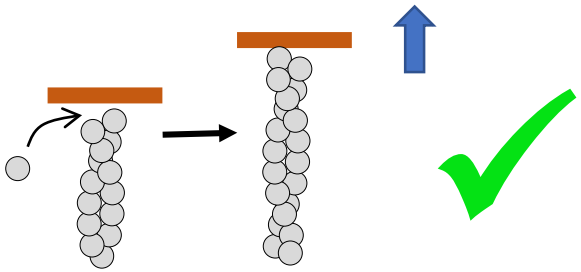
Wiesner et al, 2003

Requirement 1 : Branching generates a dense mesh of filaments

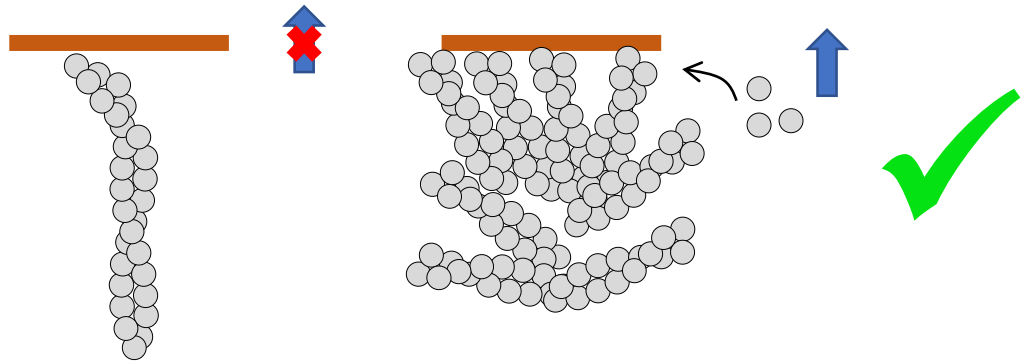


Requirements for actin propulsion

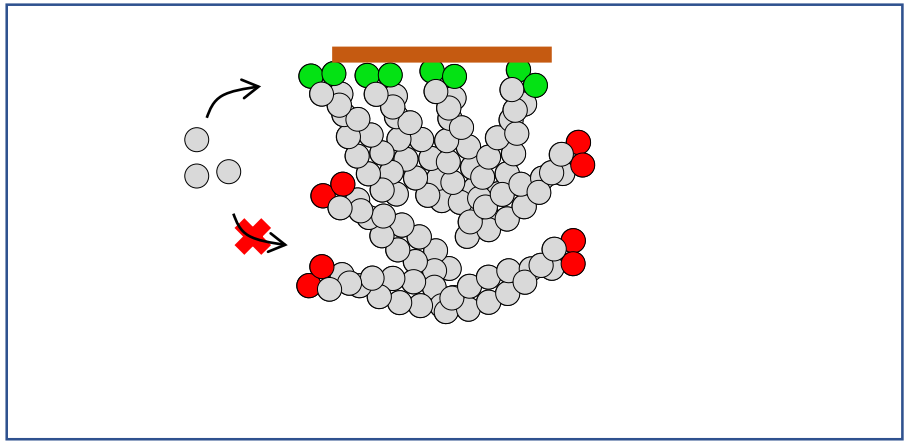
Polymerization = force



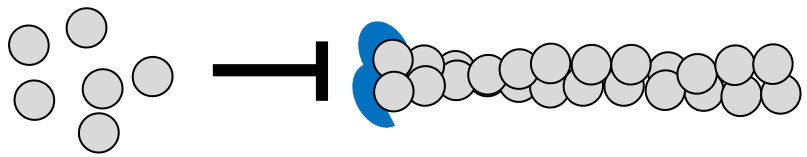
Filament mesh prevents buckling

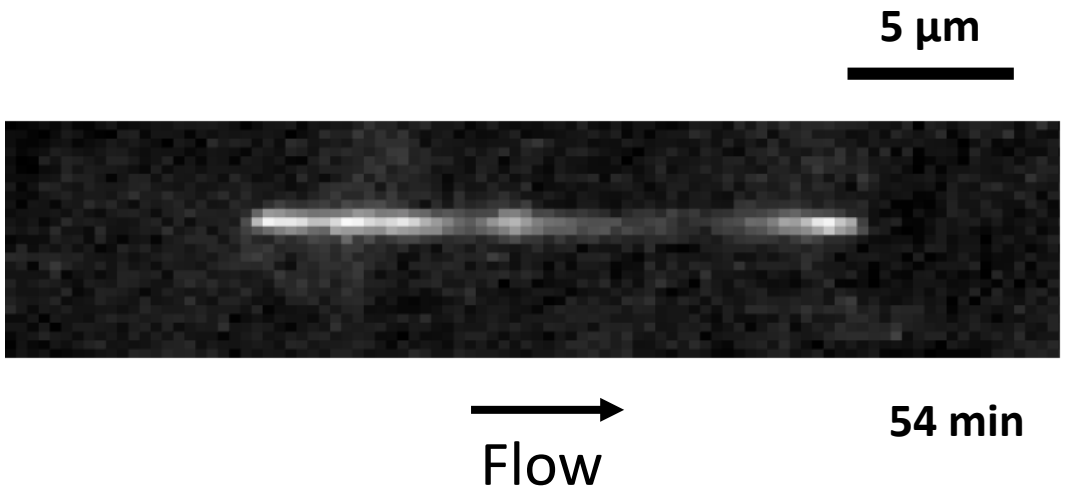


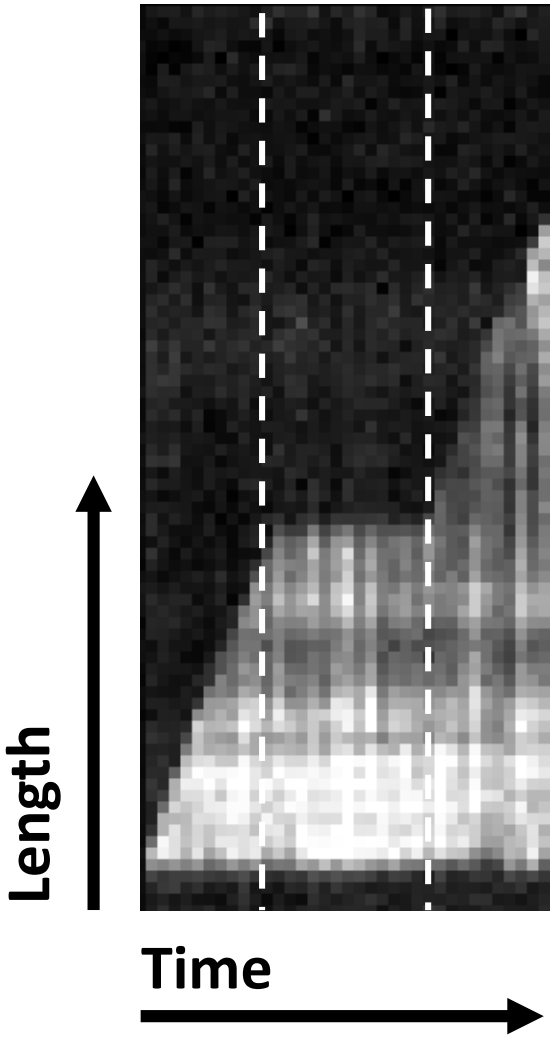
Elongate productive Filaments



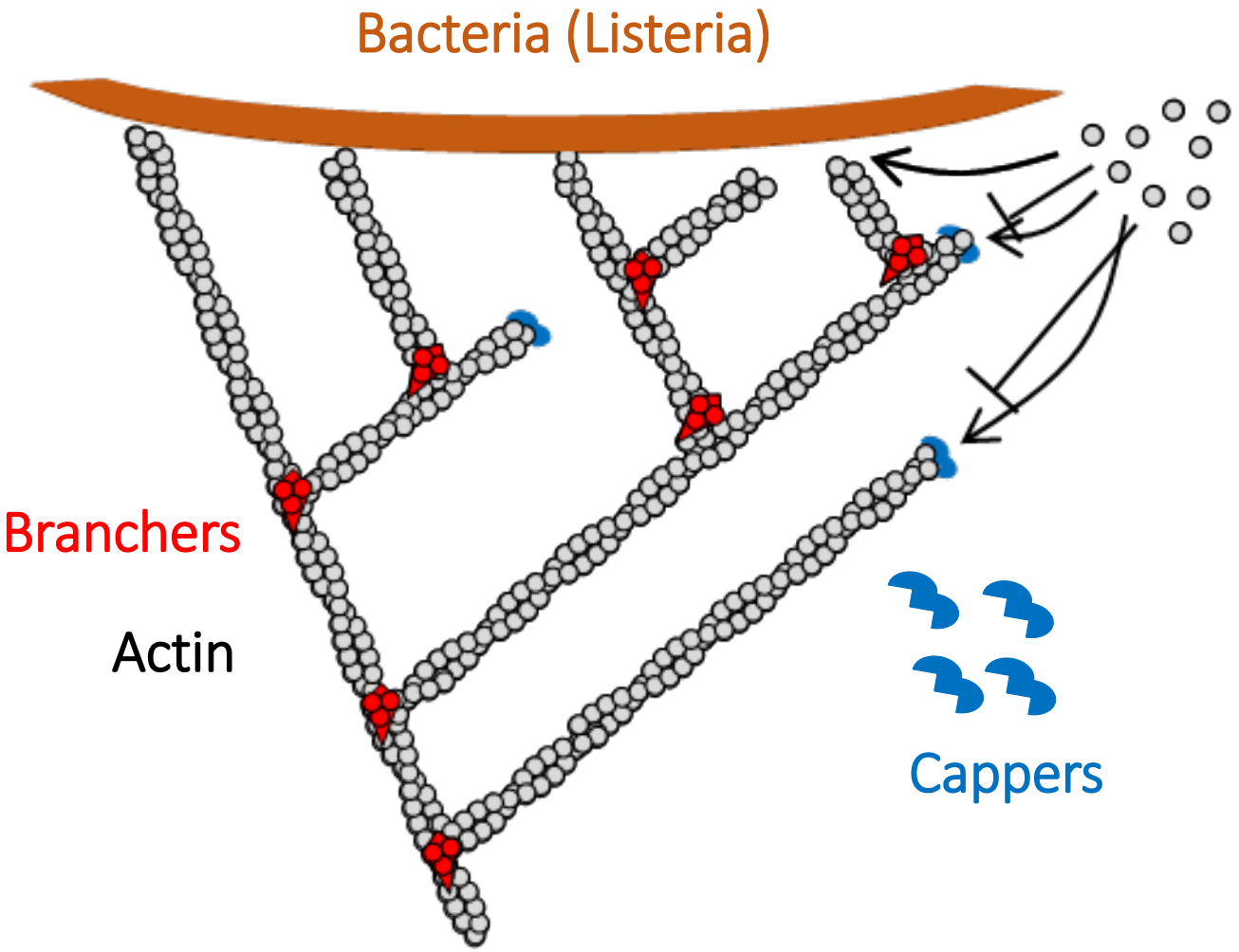
Requirement 2 : Blocking unproductive growth

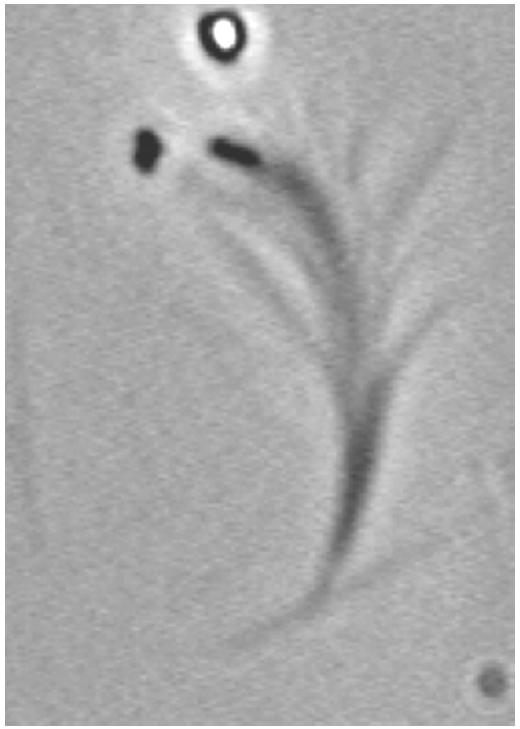




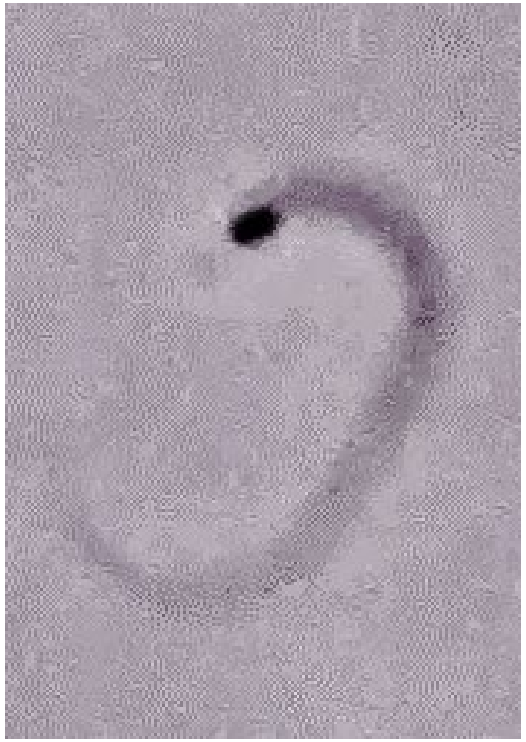


Requirement 2 : Absence of capping leads to a “fish bone” comet





Actin +
Brancher

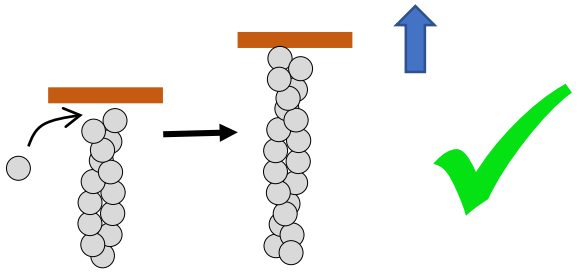


+ Cappers

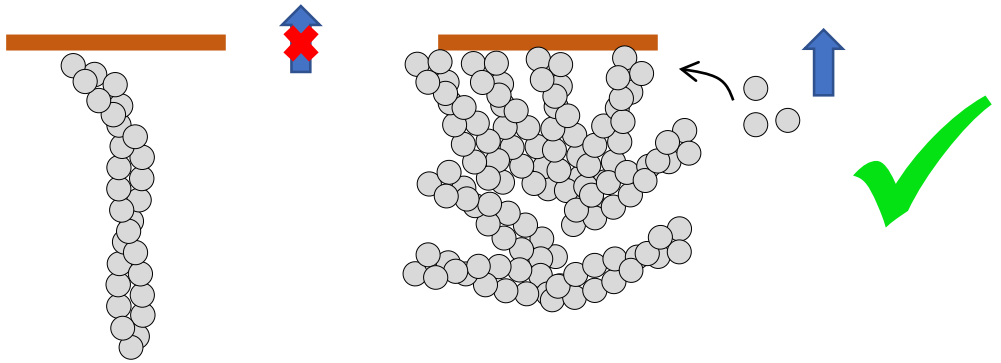
Carlier et al, 2000

Requirements for actin propulsion

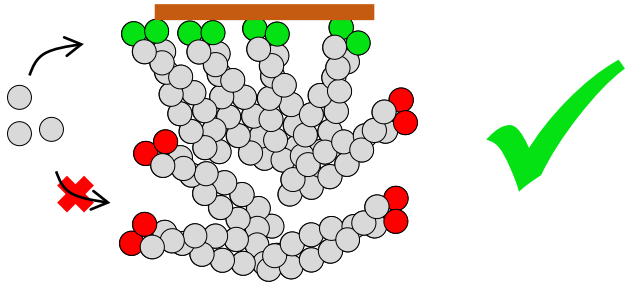
Polymerization = force



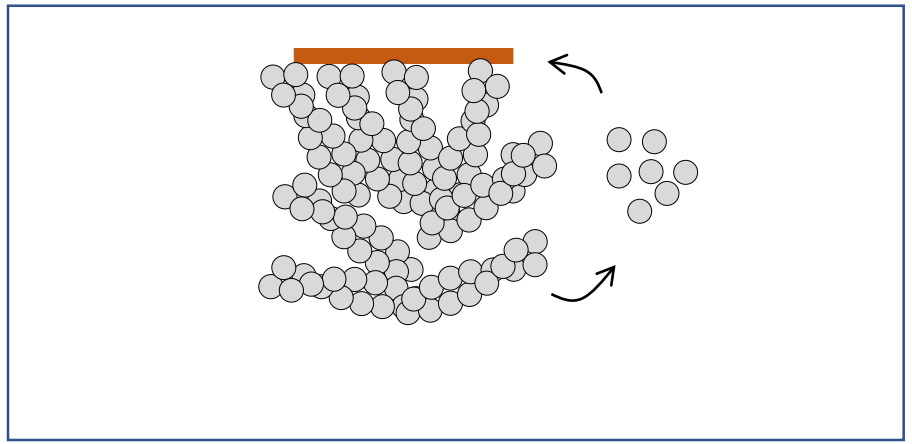
Filament mesh prevents buckling



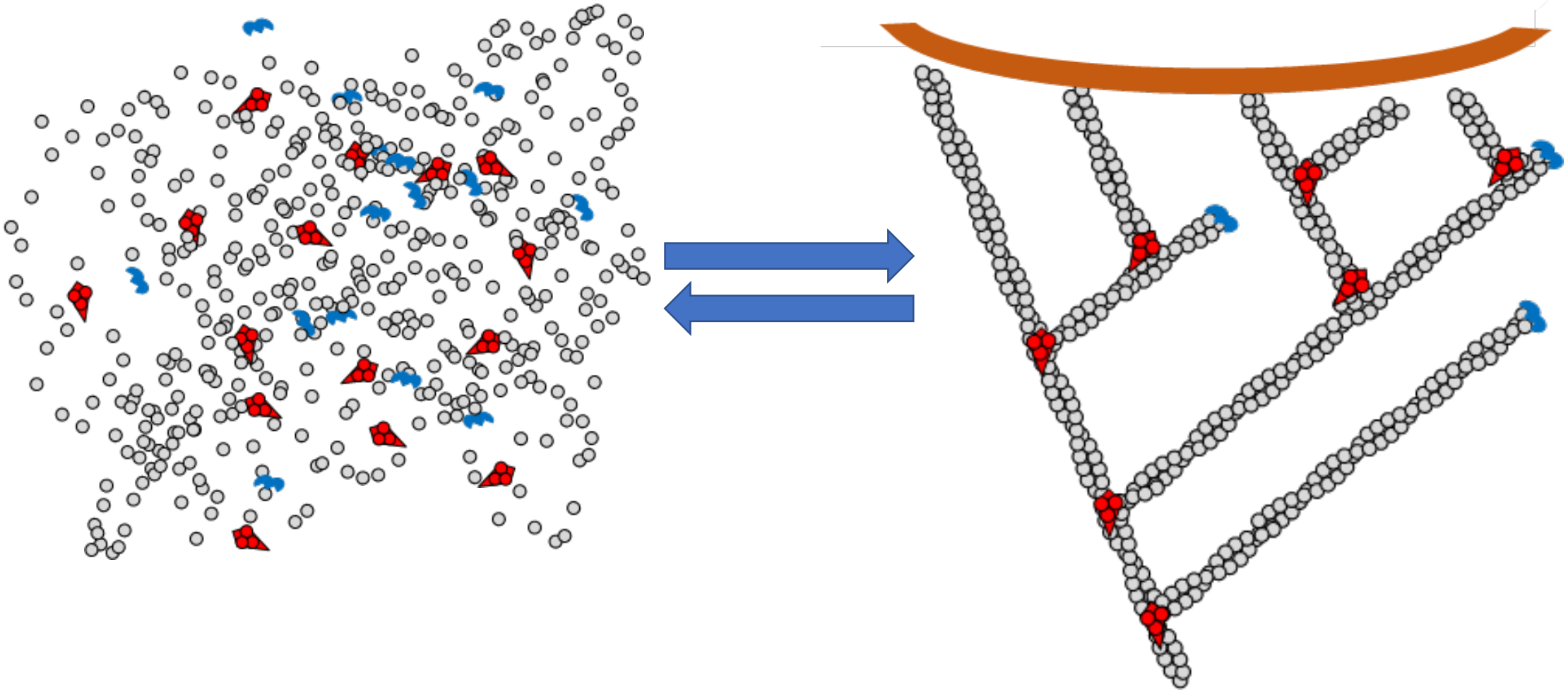
Elongate productive Filaments



Regeneration of monomers

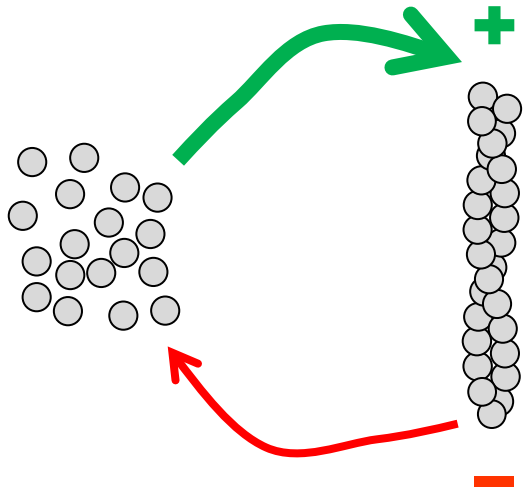


Requirement 3 : Disassembly of the network & monomer regeneration

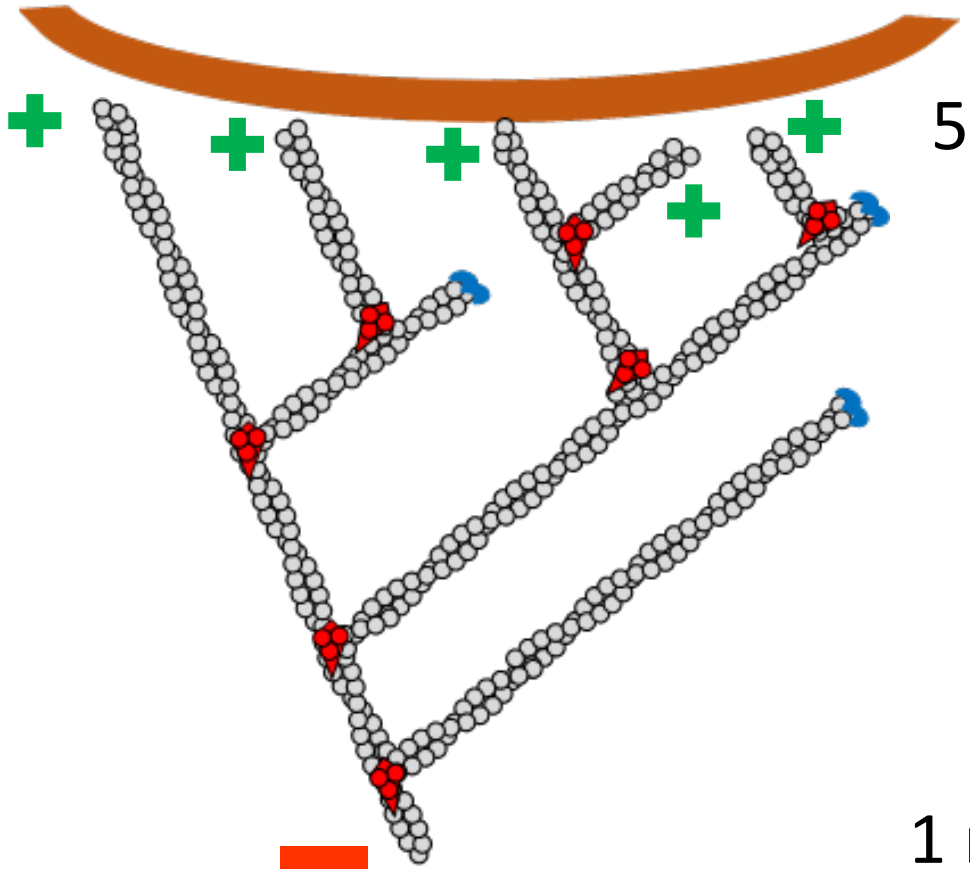


Requirement 3 : Disassembly of the network & monomer regeneration

Fast (10 monomers/µM/s)



Slow (0.2 monomers/s)



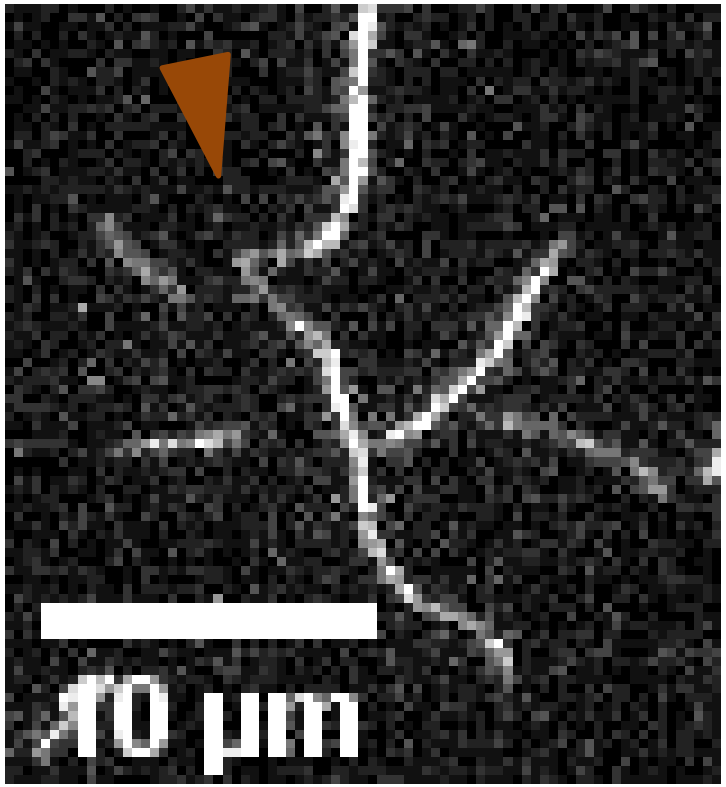
5 Plus ends

1 minus ends

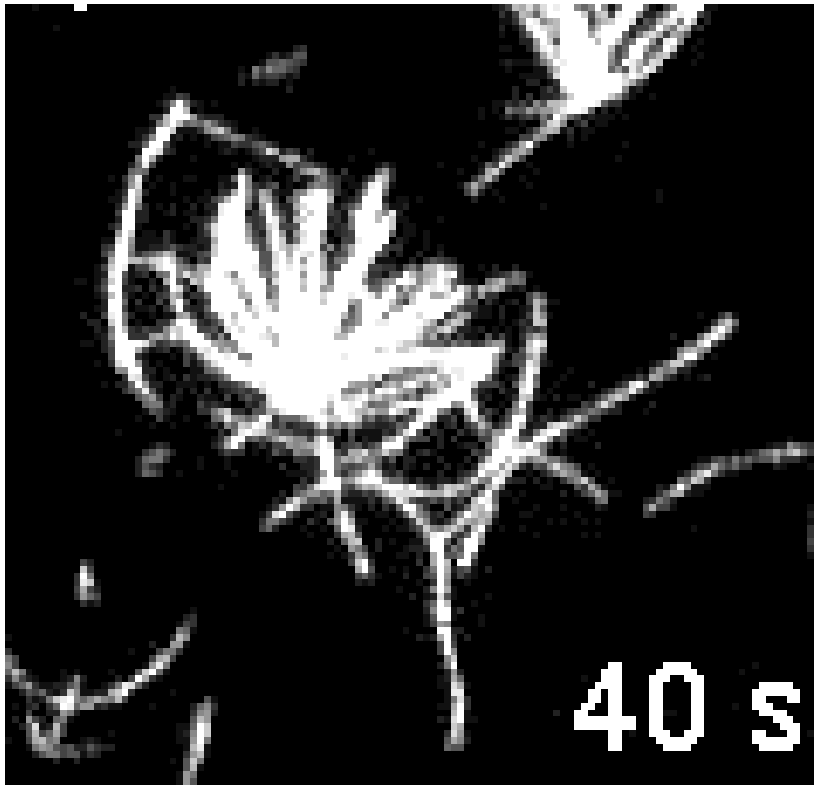
Debrancher – GMF

Actin **D**epolymerizing **F**actor (ADF)

Requirement 3 : Debrancher increases the number of minus ends

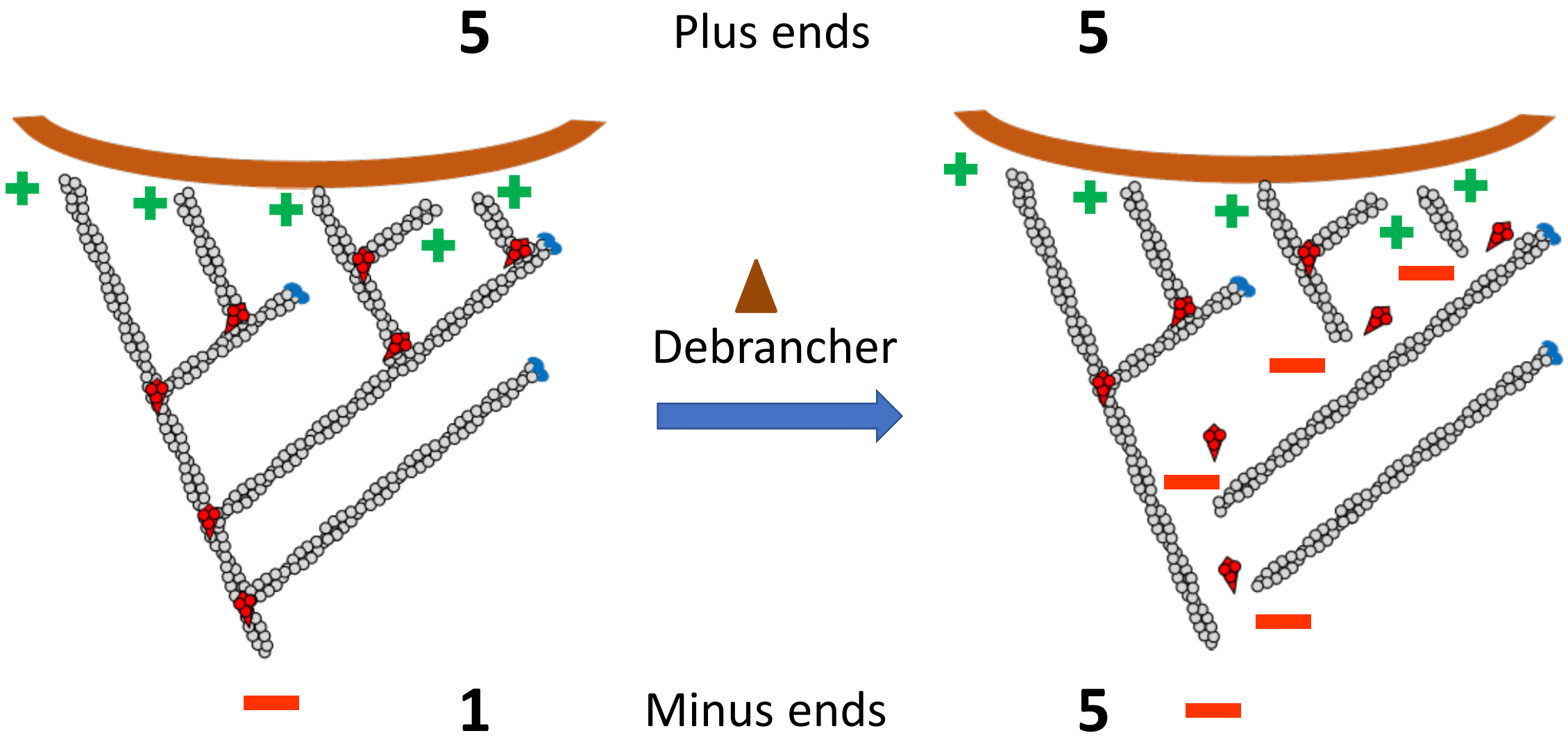


Debrancher



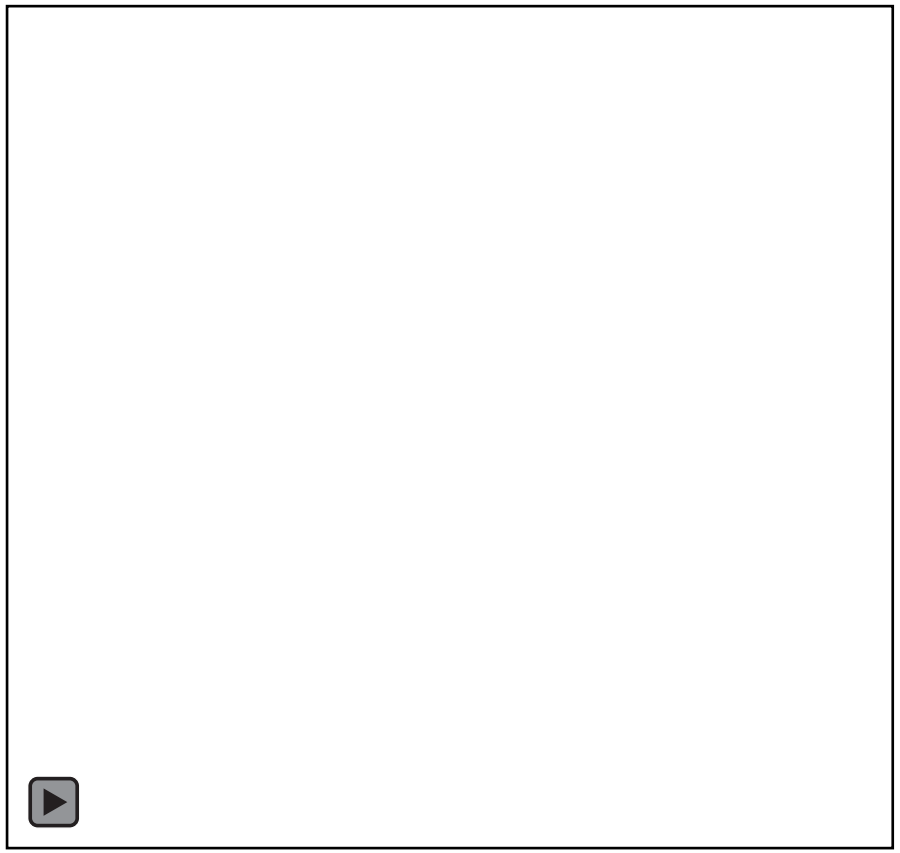
Gandhi et al, 2010; Sean Guo; Goode and Gelles labs

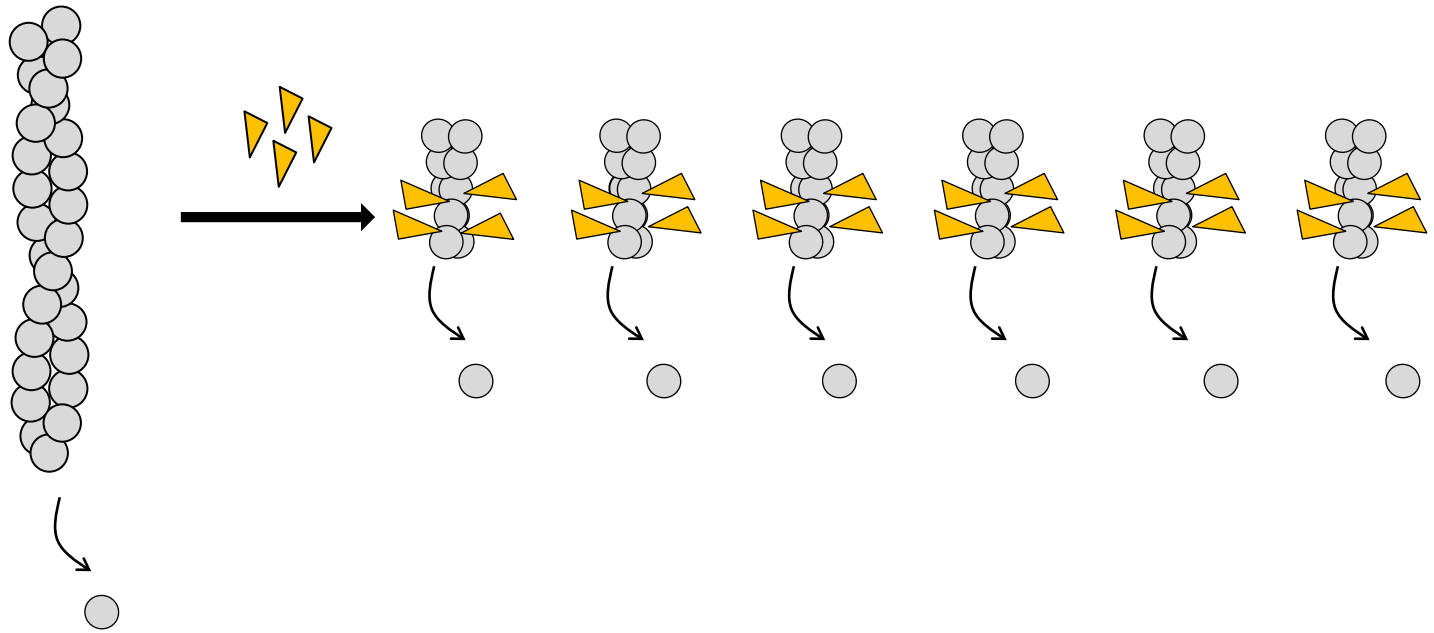
Requirement 3 : Disassembly of the network & monomer regeneration



Requirement 3 : Depolymerizer fragments actin filaments

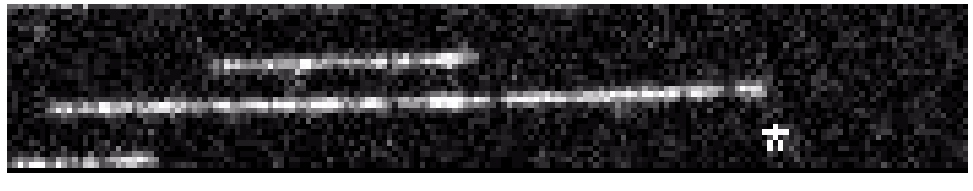
Actin + Depolymerizer

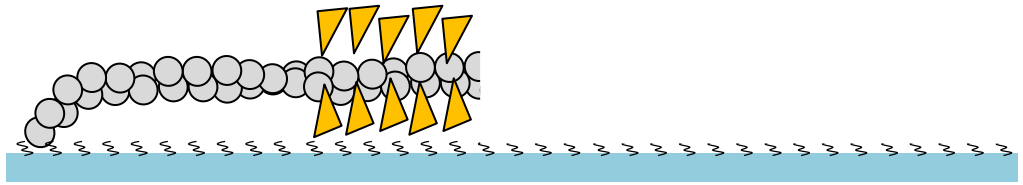


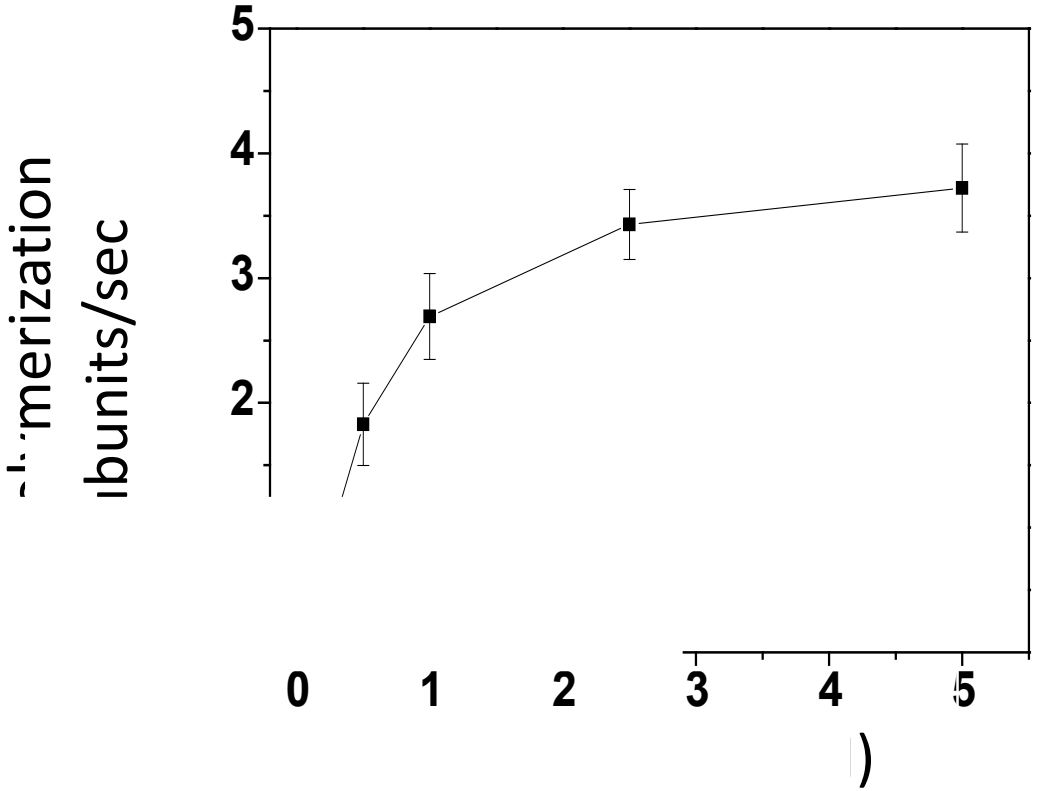


Silvia Jansen

Requirement 3 : Depolymerizer also depolymerizes filaments



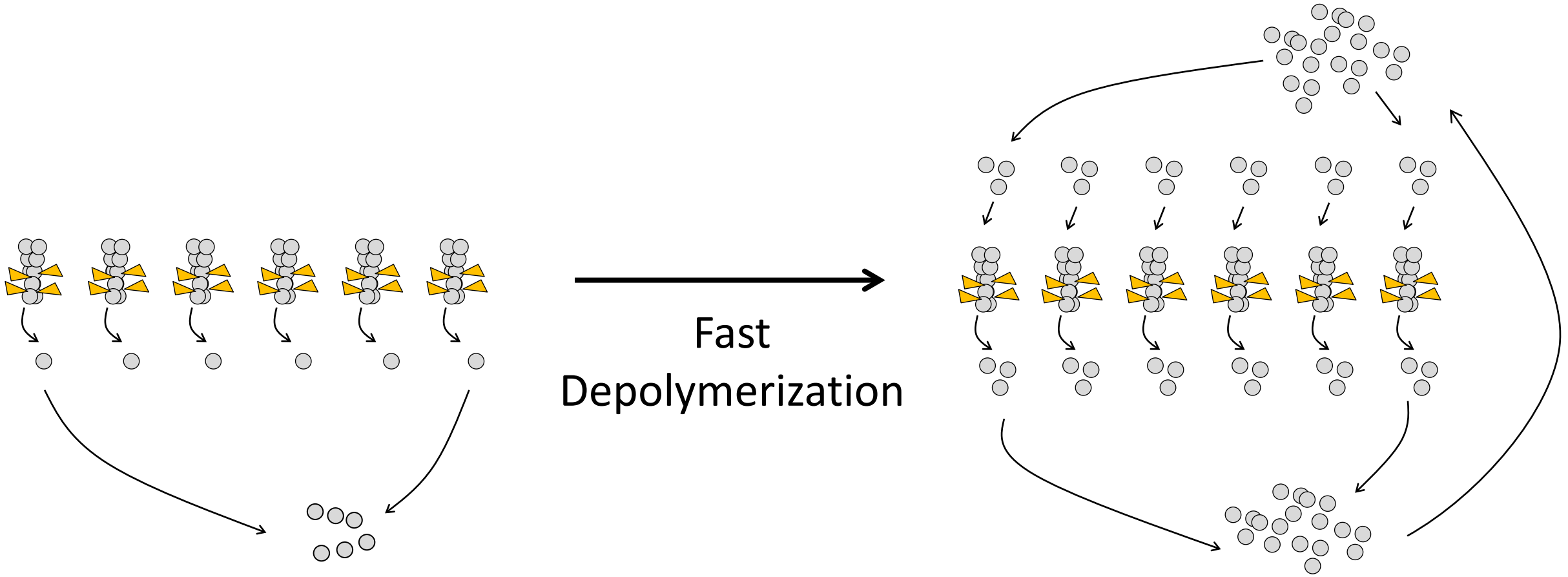




Depolymerization increases 20x

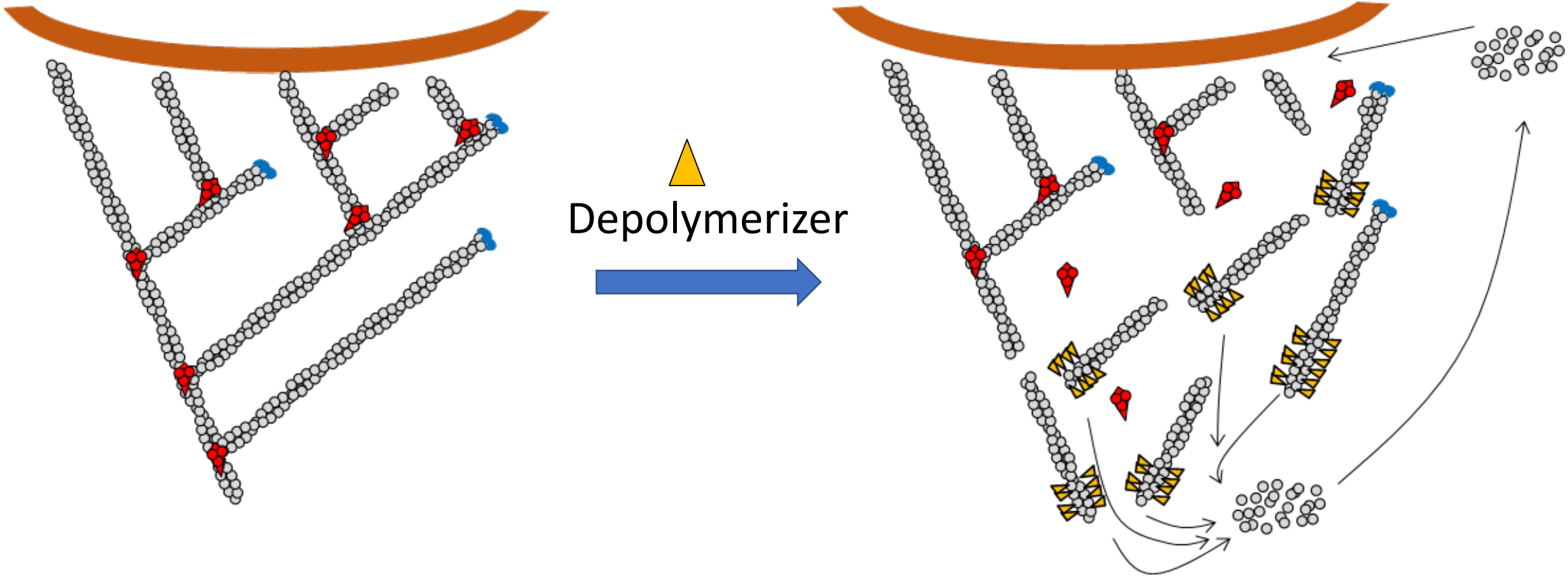
Shekhar et al, 2017

Requirement 3 : Depolymerizer fragments and depolymerizes filaments



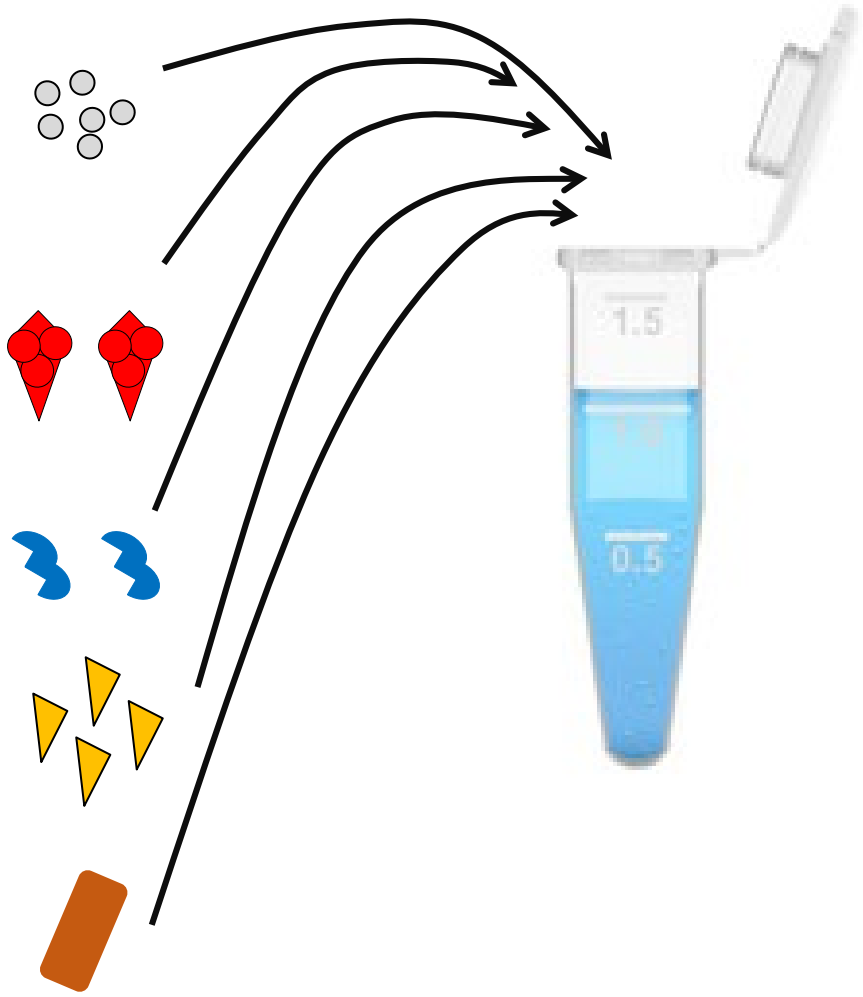
Shekhar et al, 2017

Requirement 3 : Disassembly of the network & monomer regeneration

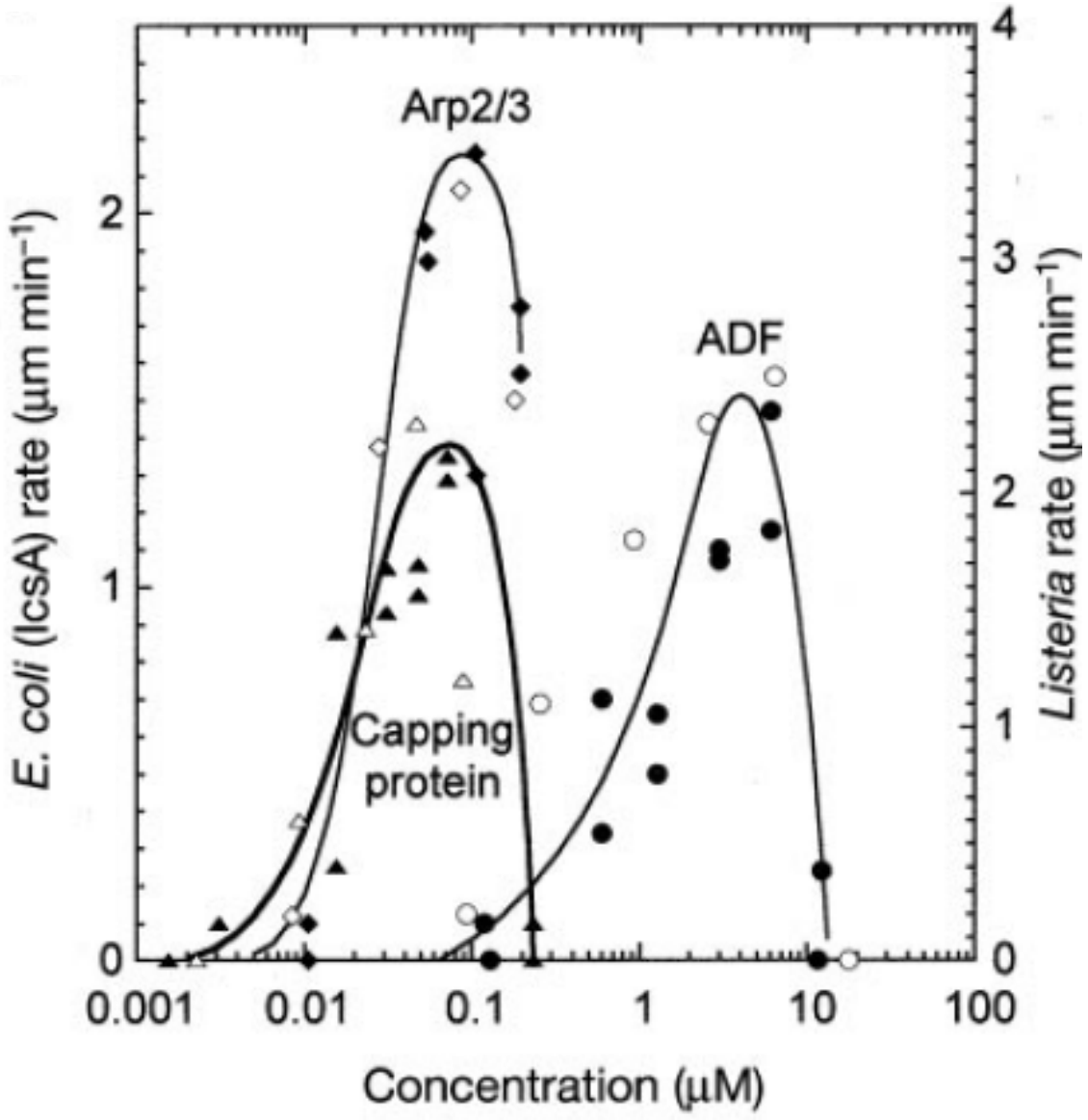


Summary

1. Actin monomers polymerize to form filaments.
2. Branchers branch filaments.
3. Cappers block filament growth.
4. Depolymerizers depolymerize.
5. Bacteria (Listeria)



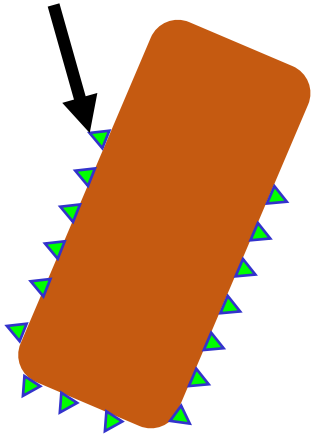
Reconstitution of Actin based motility



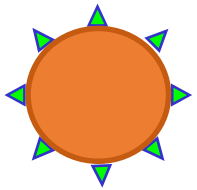
Loisel et al, 1999

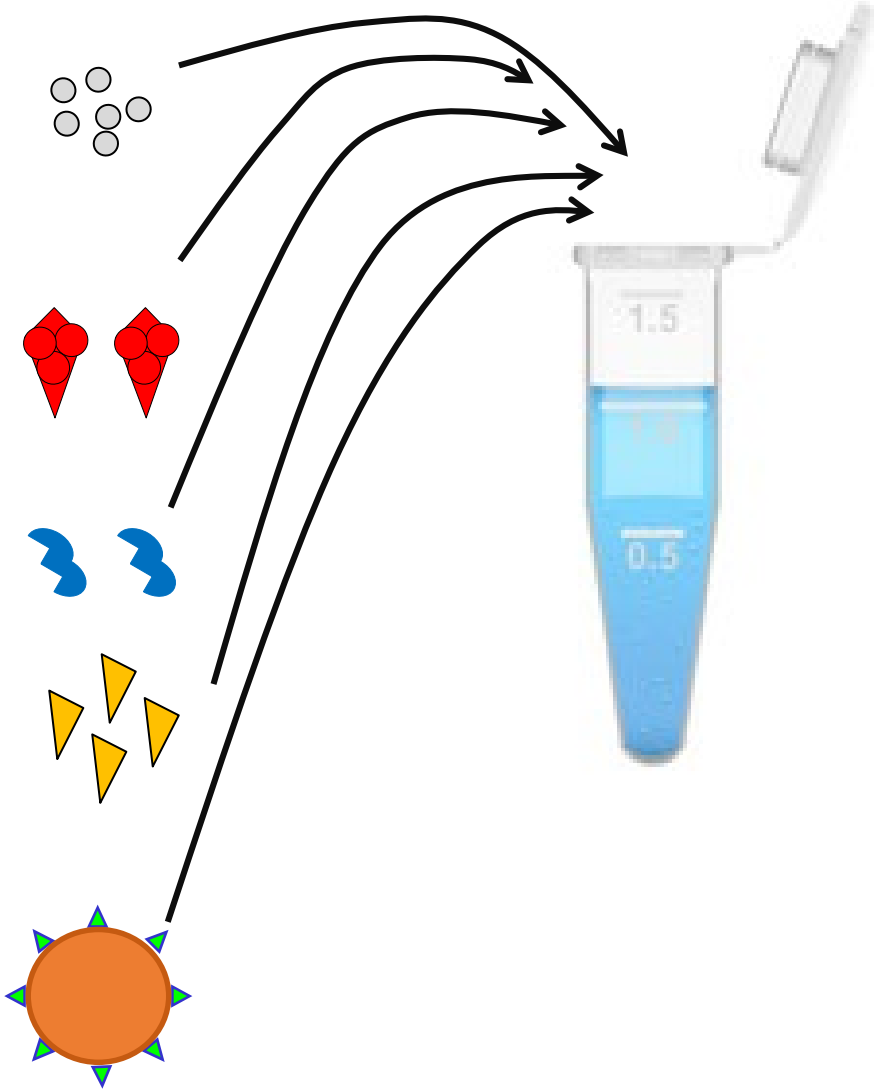
Reconstitution of Actin Based Motility

Branching activator
(ActA)

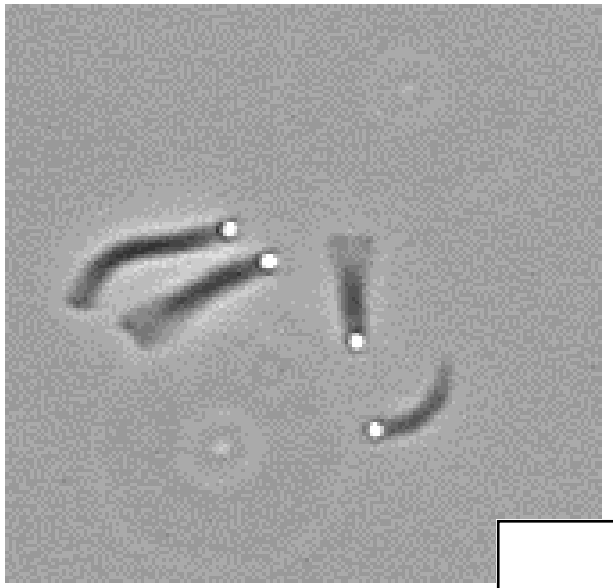


Listeria



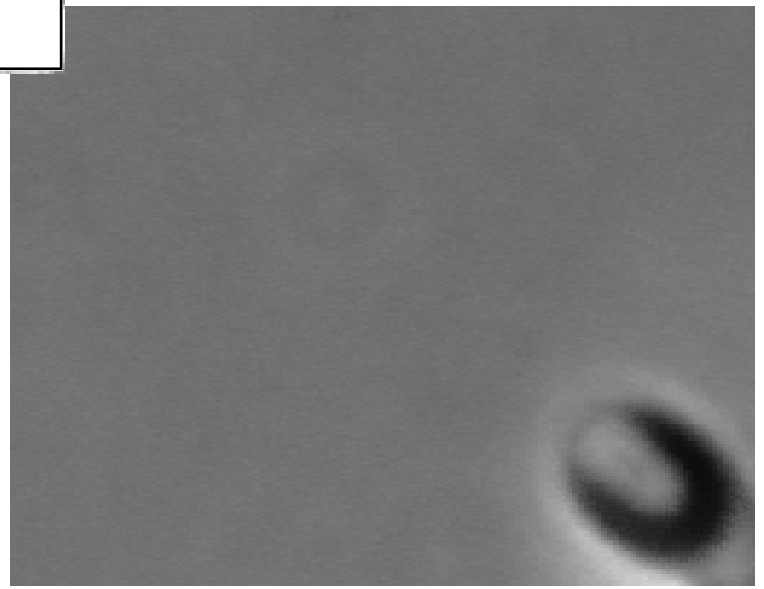


Reconstitution of Actin Based Motility



Wiesner et al, 2003

Achard et al, 2010



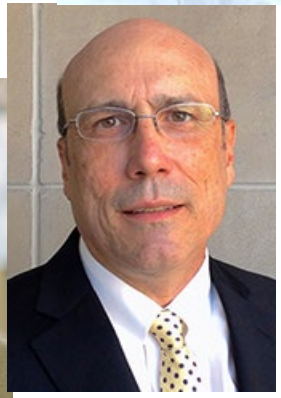
Delatour et al, 2008

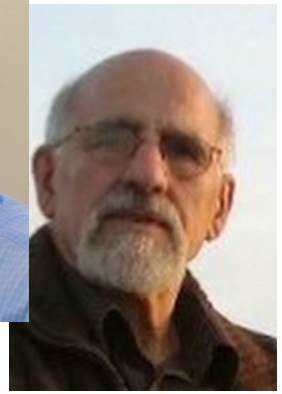
Truly an international and interdisciplinary effort

Bruce Goode

Jeff Gelles

Jané Kondev





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