

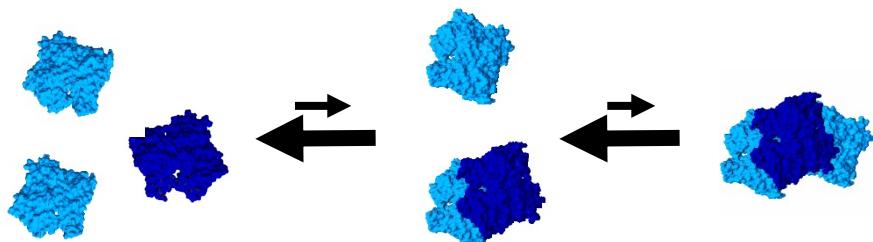
aLENS: Semi-flexible and growing filaments

BPM software summer workshop
Dimitris Vavylonis



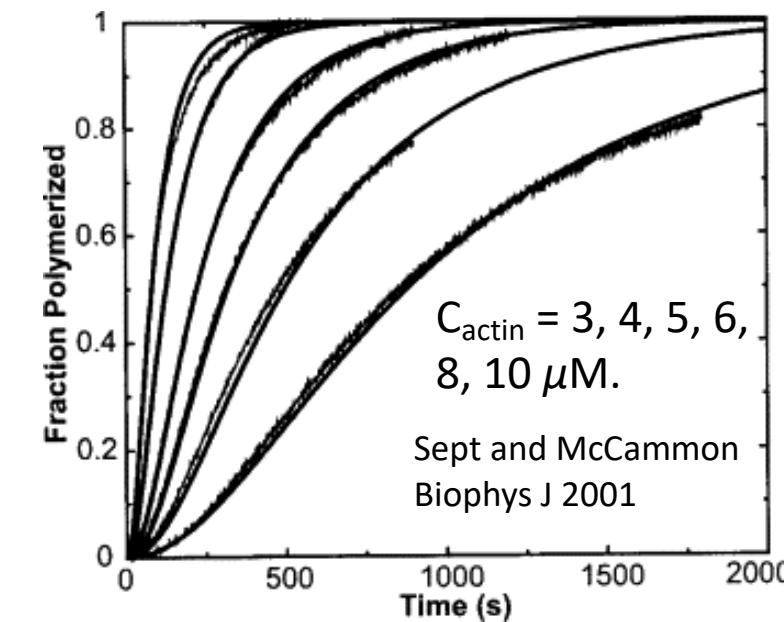
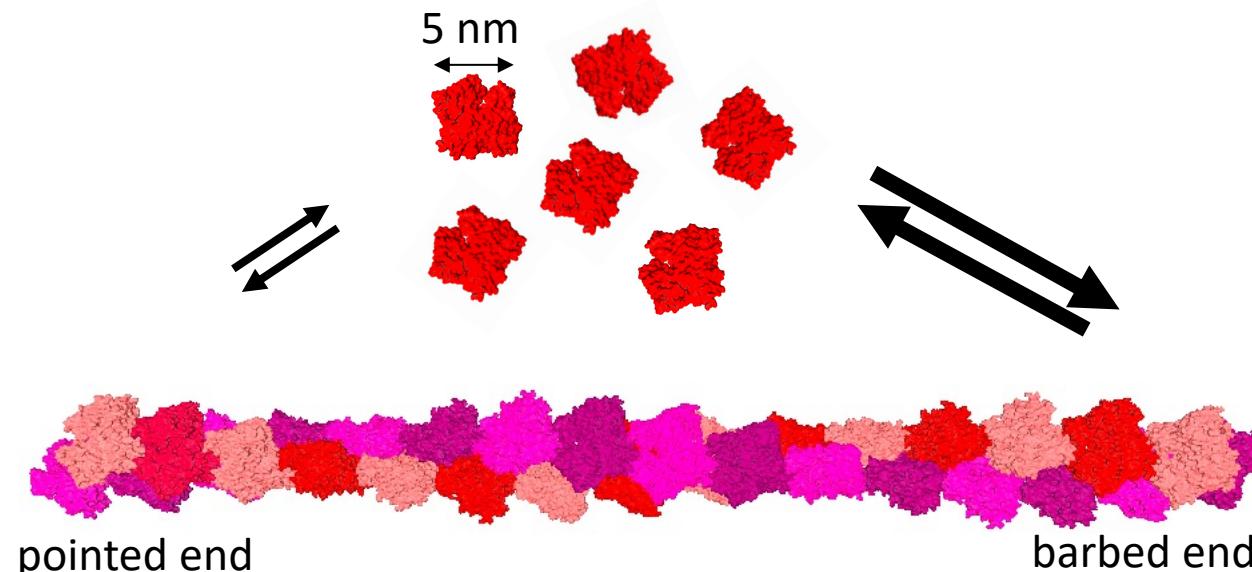
Nucleation and elongation of actin filaments in vitro and in cells

Slow filament nucleation:



Oosawa and Asakura 1975

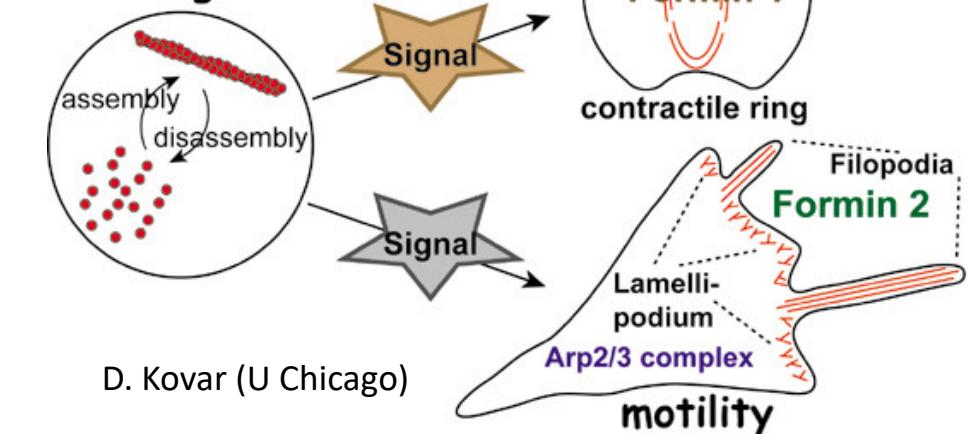
G-actin to F-actin transition:



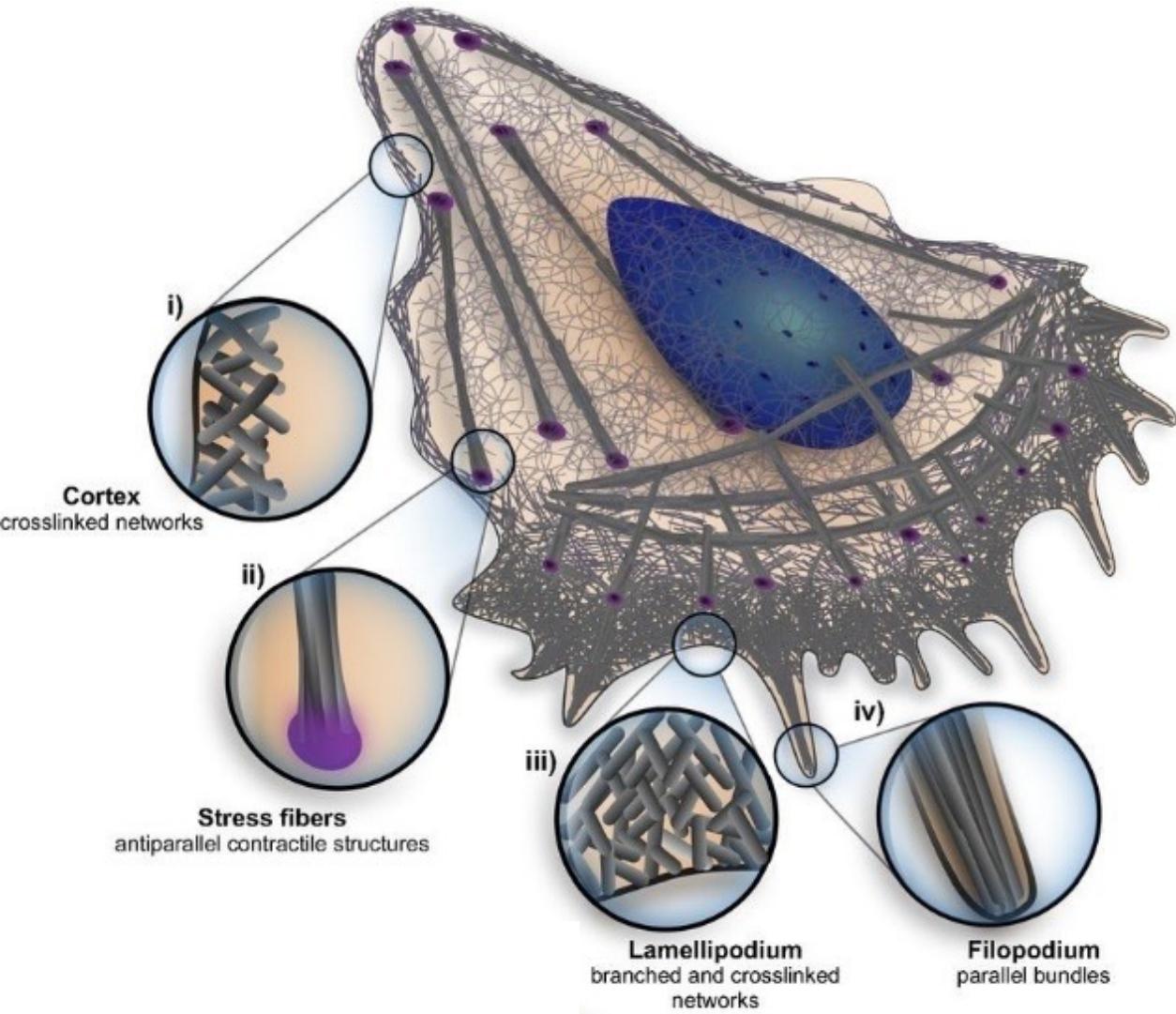
Regulation in in cells:

$C_{\text{actin}} \sim 30-1000 \mu\text{M}$

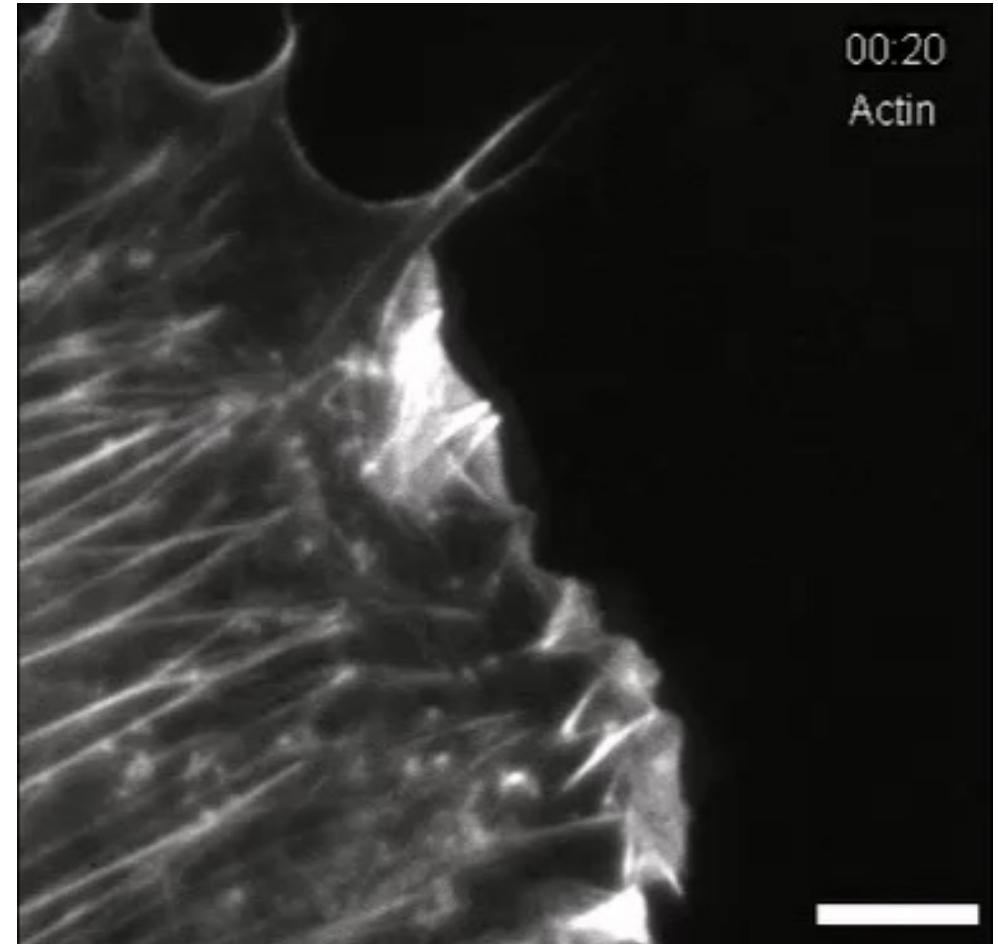
resting cell



Crawling cell motion and the actin cytoskeleton



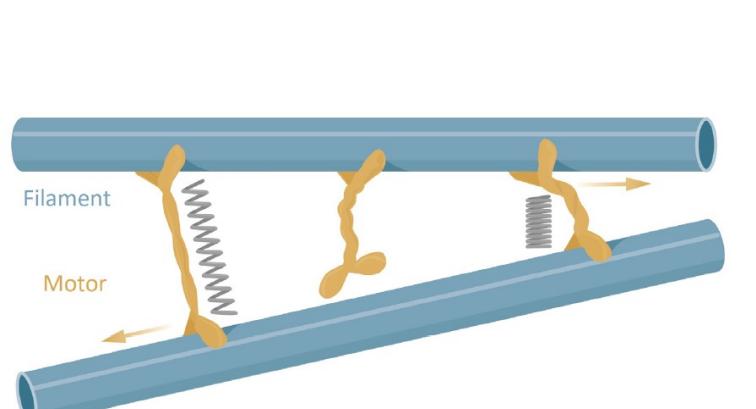
Blanchoin et al. Physiol. Reviews 2013



Fibroblast (mCherry-actin)
Nemethova, Auinger, Small JCB 2008

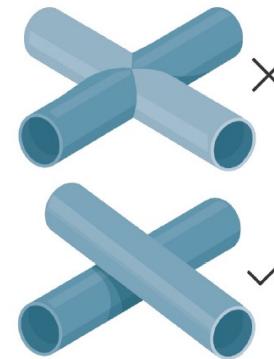
- Assembly of actin network at the leading edge
- Bundling, contraction, disassembly
- Retrograde flow

aLENS developed for high performance computing

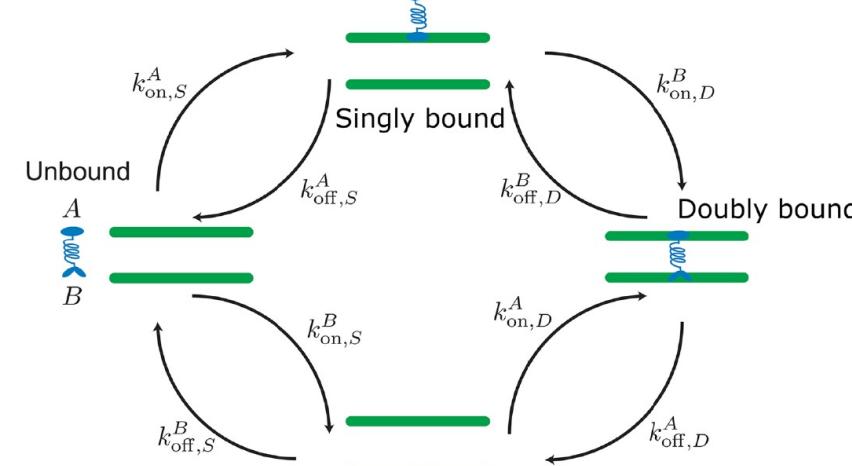


Yan et al. eLife (2021)

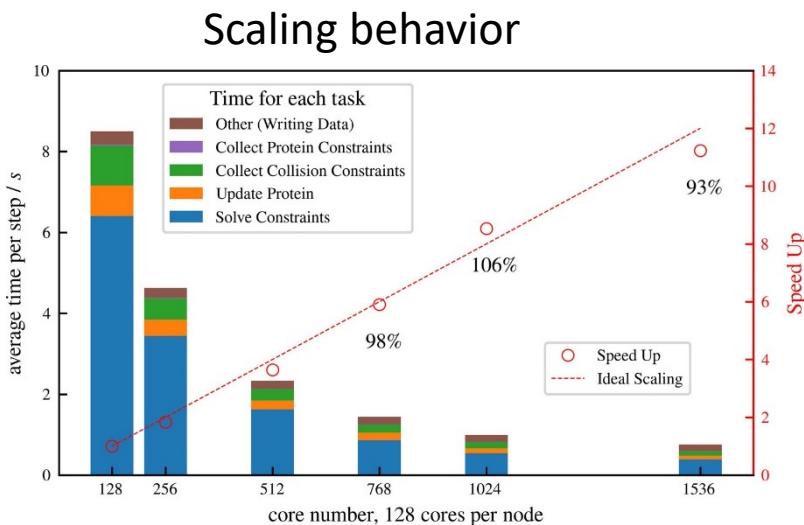
hard-core repulsion



- Constraint method to enforce hard-core repulsion between spherocylinders
- Steric interactions and crosslinking forces are incorporated in a unified implicit constraint solver.
- Brownian dynamics



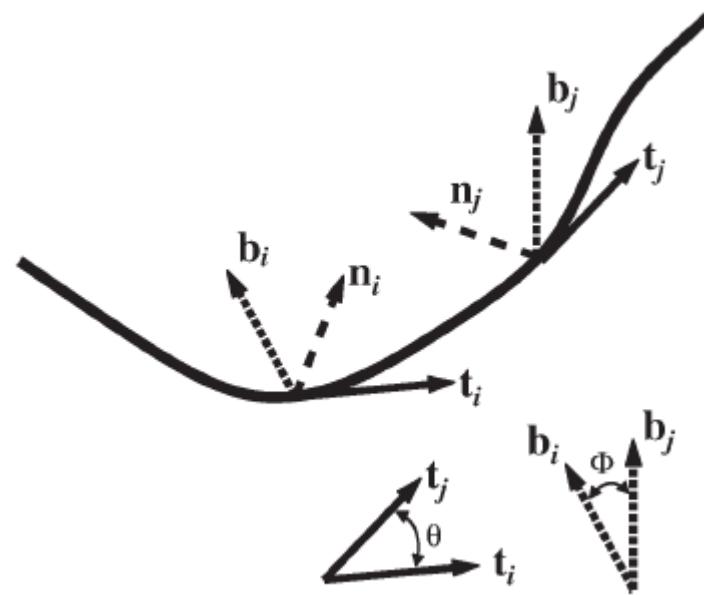
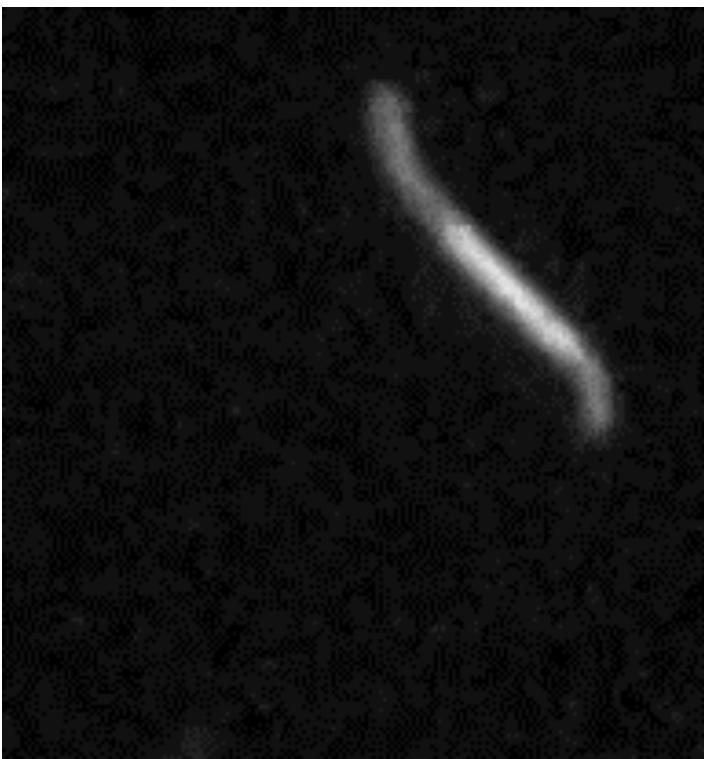
- Explicit active/pассиве crosslinking with Monte Carlo kinetics
- Detailed balance



Extension: account for filament flexibility and polymerization over time

Brownian fluctuations of actin filaments

Two bundled actin filaments fluctuating due to Brownian motion



$$\mathbf{t} = \frac{d\mathbf{r}}{ds} \quad \kappa = \left| \frac{d\mathbf{t}}{ds} \right|$$

Ward et al. *Nature Mat.* (2015)

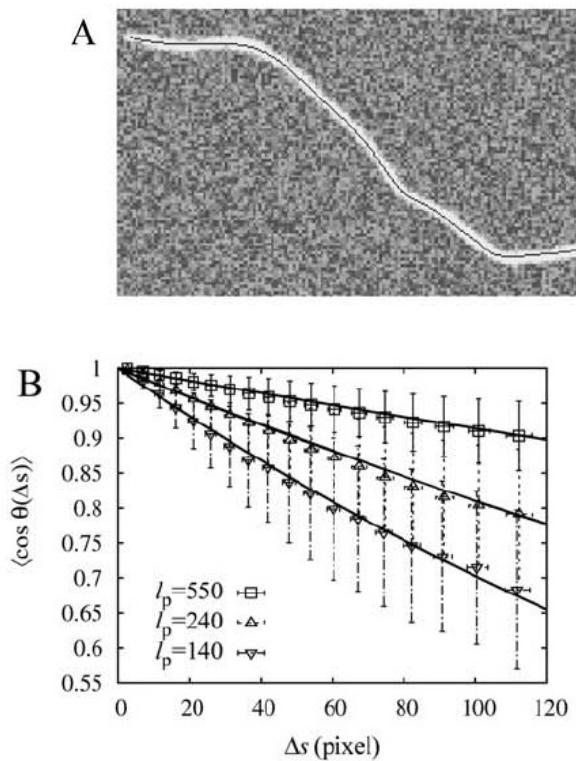
$$\langle \cos \theta(\Delta s) \rangle = \langle \mathbf{t}(s + \Delta s) \cdot \mathbf{t}(s) \rangle$$

Bending rigidity and persistence length

$$H_{\text{bending}} = \frac{b}{2} \int ds [\kappa(s)]^2$$

Persistence length $l_p = b/k_B T$

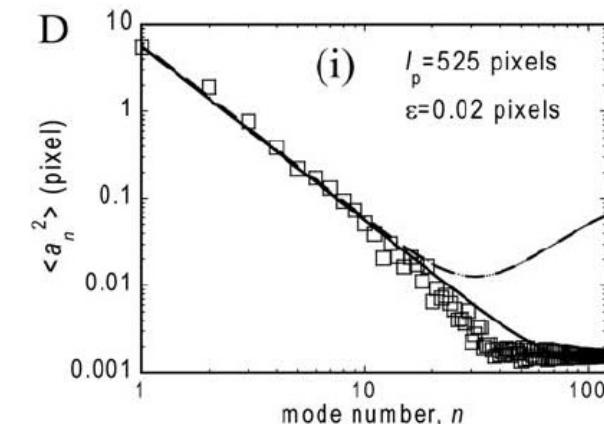
$$\langle \mathbf{t}(s + \Delta s) \cdot \mathbf{t}(s) \rangle = \exp \left\{ -\frac{(d-1)\Delta s}{2l_p} \right\}$$



Curvature distributions: $P_{\text{2D}}(\kappa) = \sqrt{\frac{2l_p \Delta s_c}{\pi}} \exp \left\{ -l_p \Delta s_c \kappa^2 / 2 \right\}$

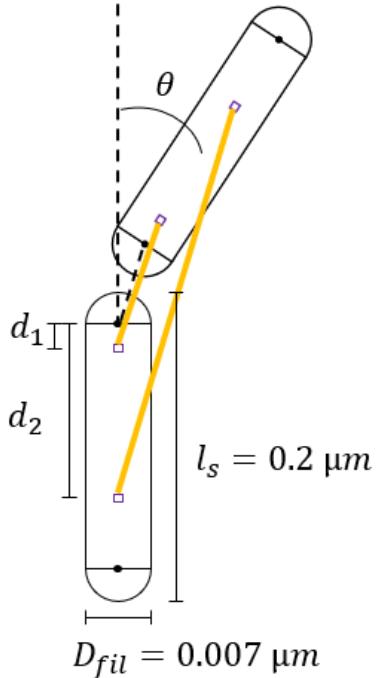
Amplitude of Fourier modes:

In 2D: $a_n = \sqrt{2/L} \int_0^L ds \theta(s) \cos(n\pi s/L)$, $\langle a_n^2 \rangle = \frac{1}{l_p} \left(\frac{L}{n\pi} \right)^2$



Implementing bending flexibility with added springs

- Use same aLENS solver to evolve rods and springs
- Add connecting springs (orange) between segments of length l_s to reproduce bending elasticity with persistence length l_p



- Assuming no gap between cylinders and matching leading bending energy terms at small θ
 \rightarrow spring constant of secondary linker for desired l_p
 \rightarrow compressed

$$k_2 \approx \frac{2k_B T l_p}{l_s} \left[\frac{1}{(d_2 + D_{fil}/2)l_0 - 2(d_2 + D_{fil}/2)^2} \right]$$

$$U_{bend}(\theta) = \frac{k_B T l_p}{2l_s} \theta^2 \text{ (small } \theta\text{)}$$

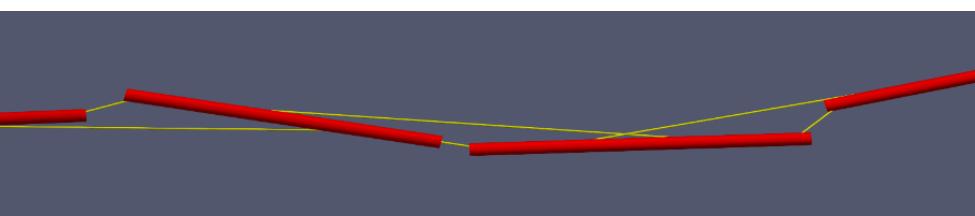
- To counter effect of compressive secondary bonds, primary linkers should be under tension such that cylinders just touching in equilibrium.

They must be placed a bit inside the segment according to aLENS spring energy

$$U_{protein}(l) = \frac{k}{2} (l - l_0 - D_{fil})^2$$

Eq. 22, from aLENS
Appendix

actin filament with 0.2 μm rod segments



Primary Protein Linker
(under tension)

$$\begin{aligned} l_{0,1} &= 0.01 \mu m \\ k_1 &= 500 pN/\mu m \\ d_1 &= 0.01 \mu m \end{aligned}$$

Secondary Protein Linker
(under compression)

$$\begin{aligned} l_{0,2} &= 0.323 \mu m, \text{ from 1.1} = \frac{l_{0,2}}{2d_2 + D_{fil}} \\ k_2 &= 92 pN/\mu m, \text{ from Eq. 1 for } l_p = 10 \mu m \\ d_2 &= 0.1465 \mu m \text{ (of order } l_s \text{ to allow smaller } k_2) \end{aligned}$$

Checking persistence length



Use time step smallest than relaxation time of springs:

$$R \sim 0.2 \text{ } \mu\text{m} \text{ (rod segment)}$$

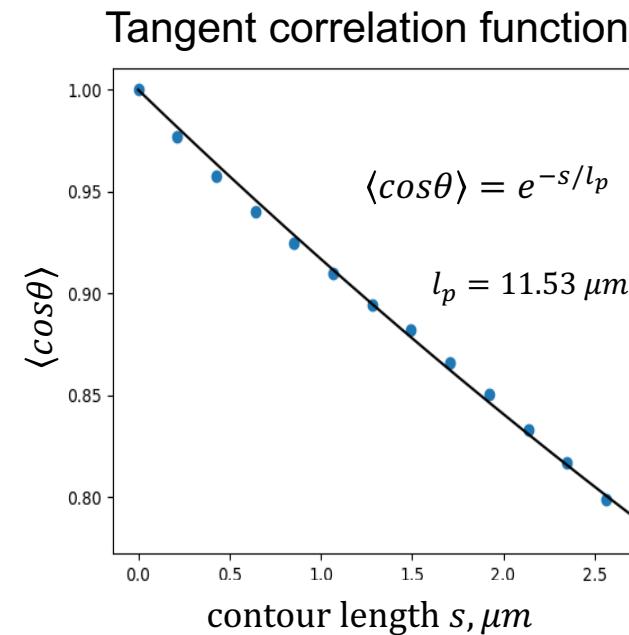
$$\eta \sim 0.01 \text{ pN}/\mu\text{m}^2\text{s}$$

$$k \sim 1000 \text{ pN}/\mu\text{m}$$

$$\tau = \frac{6\pi\eta R}{k} \sim 4 \cdot 10^{-5} \text{ s}$$

Brownian displacement of rods per time step $\langle D_{\text{fil}} \rangle \rightarrow dt \sim 10^{-5} \text{ s}$

Use time step $\sim 10^{-5} \text{ s}$



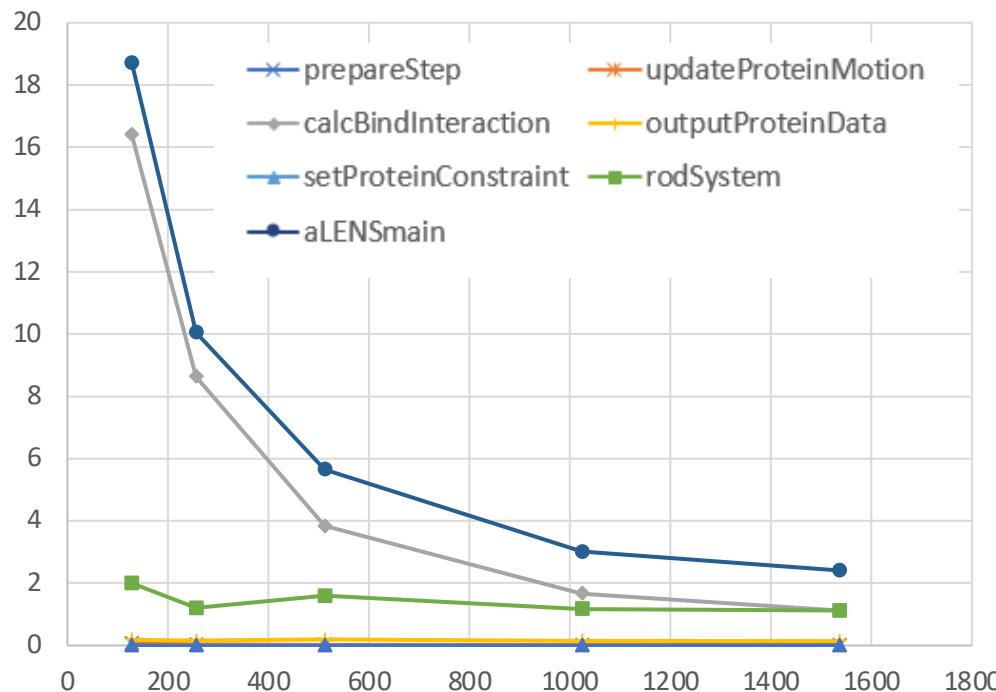
Note: Cross-links allowed between successive rod segments on same filament

Checking parallelization efficiency

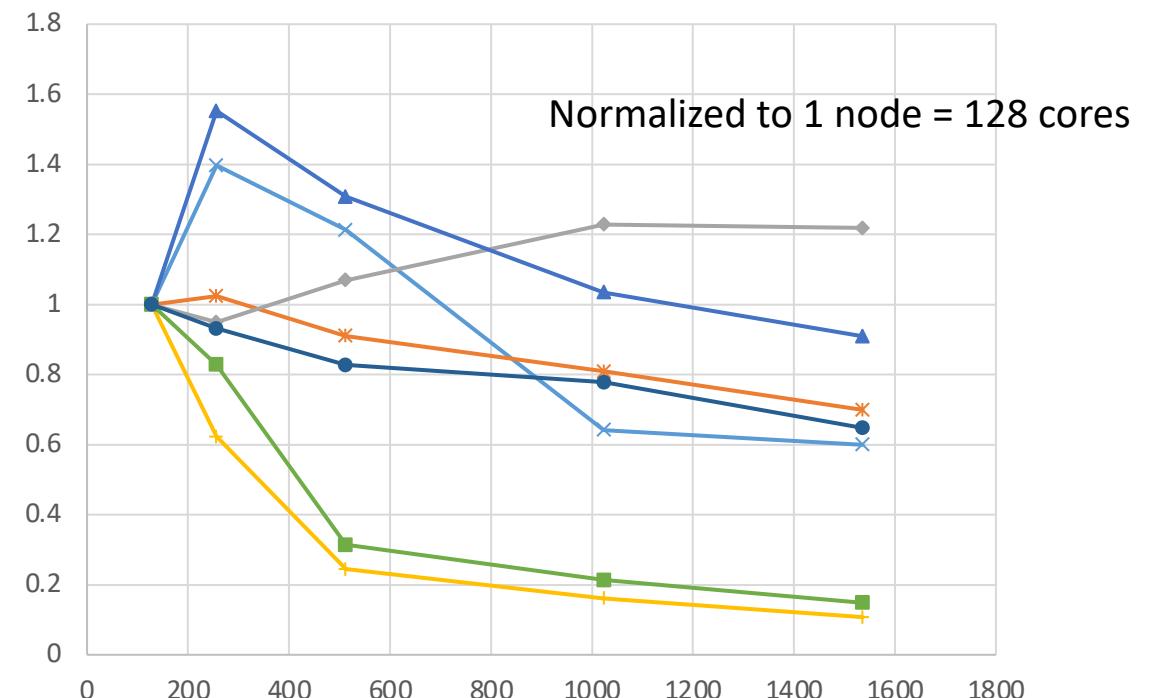
10^4 filaments of 10 segments connected by two permanently-bound protein linkers to neighbors (4 xlink total)

$dt = 10^{-5}$ s, following numbers show is average of first 100 steps with 10 data saves

Time per step (s) vs core number (4 xlink/segment)



Efficiency vs core number (4 xlink/segment)



calcBindInteraction has good scaling but overall time seems large for the task.

Fork of SimToolbox to add permanent crosslinks between filament segments

<https://github.com/vavylonis/SimToolbox>

```
// dmr518, adding in secondary linkers with hardcoded parameters and position, 20220624
// adding an secondary bilateral constraint for semiflexible filaments
// cylinders are not treated as spheres for bilateral constraints
// constraint is always added between Pp and Qm
// constraint target length is radiusI + radiusJ + runConfig.linkGap

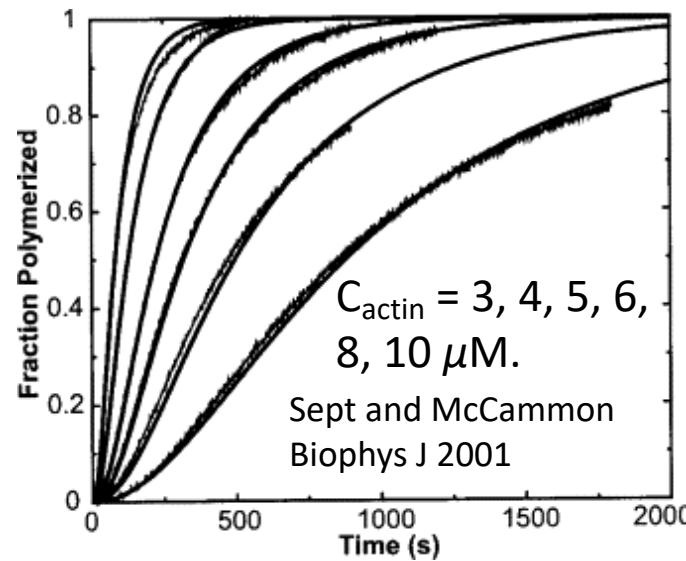
// see 20220601 presentation for these definitions
const double d2 = 0.1465; // um
const double l0_2 = 0.323; // um
const double k2 = 92; // pN/um

//const Evec3 directionI = ECmapq(syI.orientation) * Evec3(0, 0, 1);
const Evec3 Pp_secondary = centerI + directionI * (0.5 * syI.length - d2); // plus end
//const Evec3 directionJ = ECmap3(syJ.direction);
const Evec3 Qm_secondary = centerJ - directionJ * (0.5 * syJ.length - d2);
const Evec3 Ploc_secondary = Pp_secondary;
const Evec3 Qloc_secondary = Qm_secondary;
const Evec3 rvec_secondary = Qloc_secondary - Ploc_secondary;
const double rnorm_secondary = rvec_secondary.norm();
//const double delta0_secondary = rnorm_secondary - syI.radius - syJ.radius - runConfig.linkGap;
const double delta0_secondary = rnorm_secondary - syI.radius - syJ.radius - l0_2;
const double gamma_secondary = delta0_secondary < 0 ? -delta0_secondary : 0;
const Evec3 normI_secondary = (Ploc_secondary - Qloc_secondary).normalized();
const Evec3 normJ_secondary = -normI_secondary;
const Evec3 posI_secondary = Ploc_secondary - centerI;
const Evec3 posJ_secondary = Qloc_secondary - centerJ;
ConstraintBlock conBlock_secondary(delta0_secondary, gamma_secondary,           // current separation, initial guess of gamma
                                  syI.gid, syJ.gid,          //
                                  syI.globalIndex,          //
                                  syJ.globalIndex,          //
                                  normI_secondary.data(), normJ_secondary.data(), // direction of collision force
                                  posI_secondary.data(), posJ_secondary.data(), // location of collision relative to particle center
                                  Ploc_secondary.data(), Qloc_secondary.data(), // location of collision in lab frame
                                  false, true, k2, 0.0);

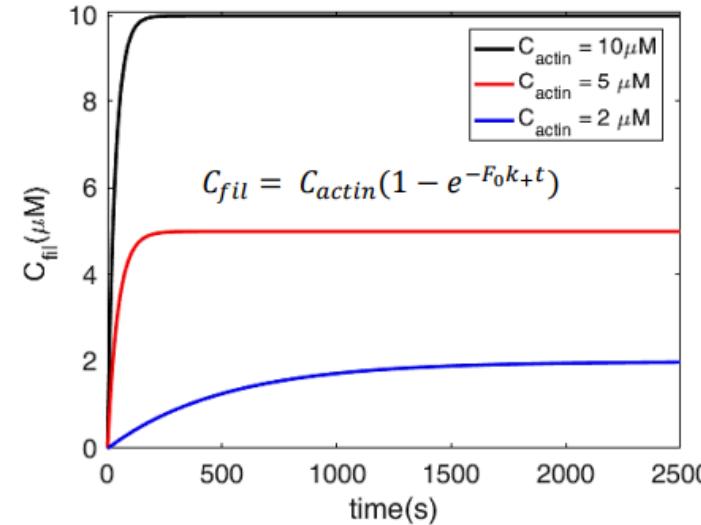
Emat3 stressIJ_secondary;
CalcSylinderNearForce::collideStress(directionI, directionJ, centerI, centerJ, syI.length, syJ.length,
                                       syI.radius, syJ.radius, 1.0, Ploc_secondary, Qloc_secondary, stressIJ_secondary);
conBlock_secondary.setStress(stressIJ_secondary);
conQue.push_back(conBlock_secondary);
```

David Rutkowski

Implementing filament elongation versus time



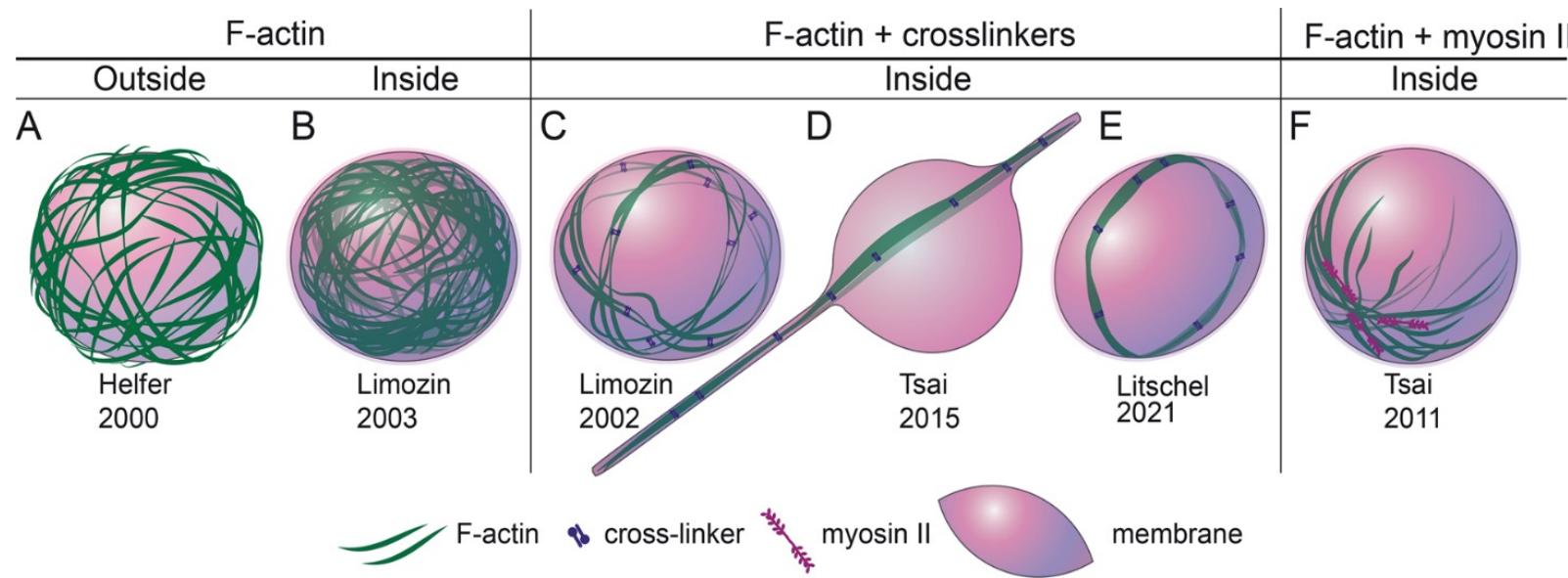
Approximate polymerization curve



```
double tAdd = 0;  
do {  
    // Formula: tadd = log(k+ c0 / (k+ c0 Exp(-F0 k+ tadd_prev) - 0.2 um * 370 * F0 * k+)/(F0 k+)  
    // F0 = 2.2 nM seeds, c0 = 5 uM actin  
    tAdd = log(50./(50./exp(0.022*tAdd) - 1.628))/0.022;  
}  
while((rodSystem.getStepCount()-1)*rodSystem.runConfig.dt > tAdd);  
  
if(rodSystem.getStepCount()*rodSystem.runConfig.dt > tAdd)  
{  
    // add cylinder to barbed ends  
}
```

Uses rodSystem.addNewSylinder

Test application: Actin filaments under confinement in vitro



dos Santos and Campillo *Biochem Soc Trans* (2022)

Experimental Challenges

- Many parameters to optimize (relative concentrations, size, surface attachment, type of linkers or motors)
- Modeling needed to classify patterns and identify optimal conditions

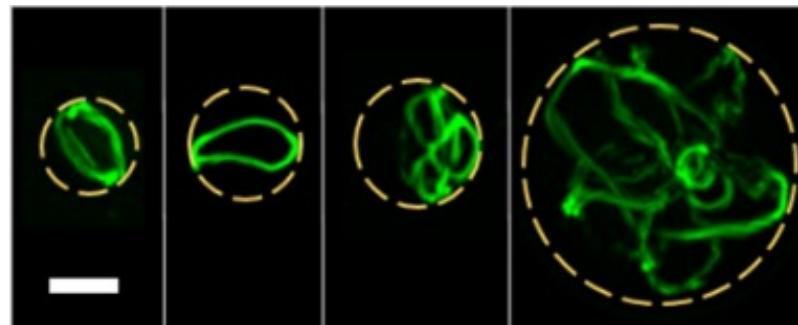
Limitations of various prior modeling approaches

- Not accounting for cross-linkers and motors explicitly in 3D
- System size often much smaller than experiment due to computational cost
- Viscosity assumed higher than many experiments
- Accounting for how network structure depends on kinetics of actin polymerization

Actin ring formation with confined actin and passive crosslinkers

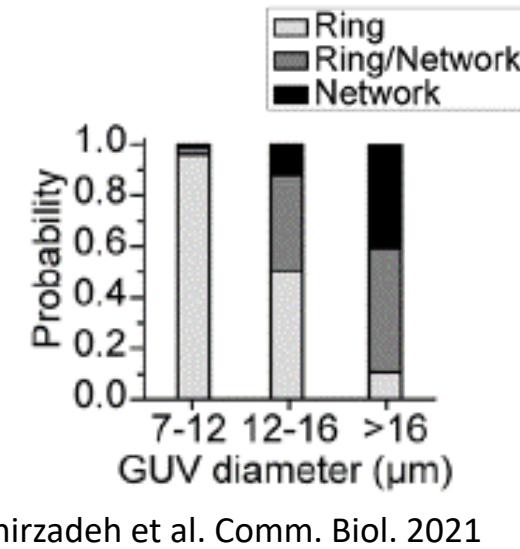
Honda, Takiguchi, Ishikawa, Hotani *J Mol. Biol.* 1999
Limozin and Sackmann, PRL 2002
Hase, Yoshikawa, *J. Chem. Phys.* 2006
Miyazaki et al. *Nature Cell Biol* 2015
Tsai and Koenderink *Soft Matter* 2015
Adeli Koudehi et al. *Cytoskeleton* 2019
Litschel et al. *Nat Comm* 2021

5 μM actin concentration + α -actinin



GUV diameter (μm)

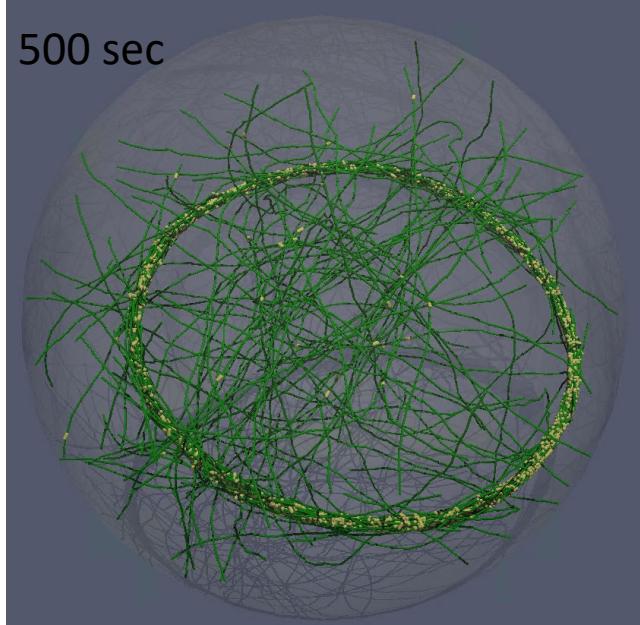
7-12 Small 12-16 Medium 16-24 Large > 24 Large



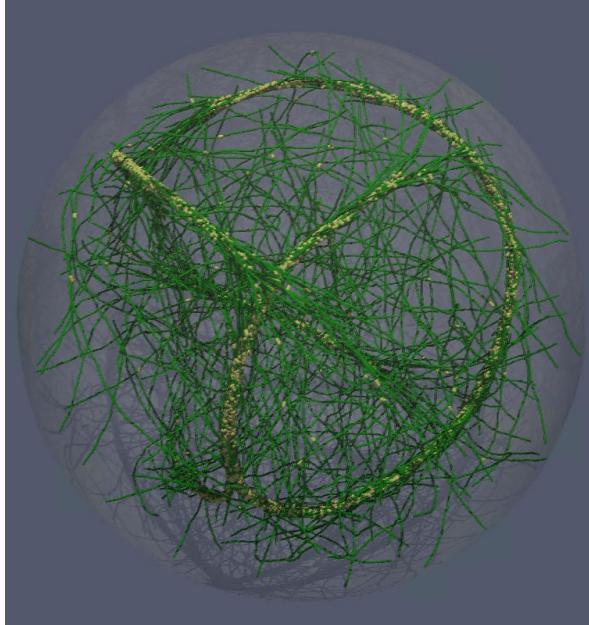
Bashirzadeh et al. *Comm. Biol.* 2021

aLENS simulations, 5 μM actin + α -actinin

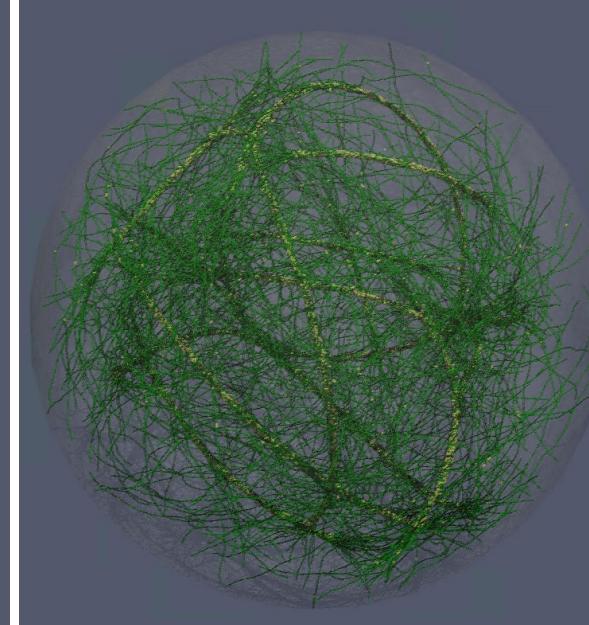
D = 6 μm



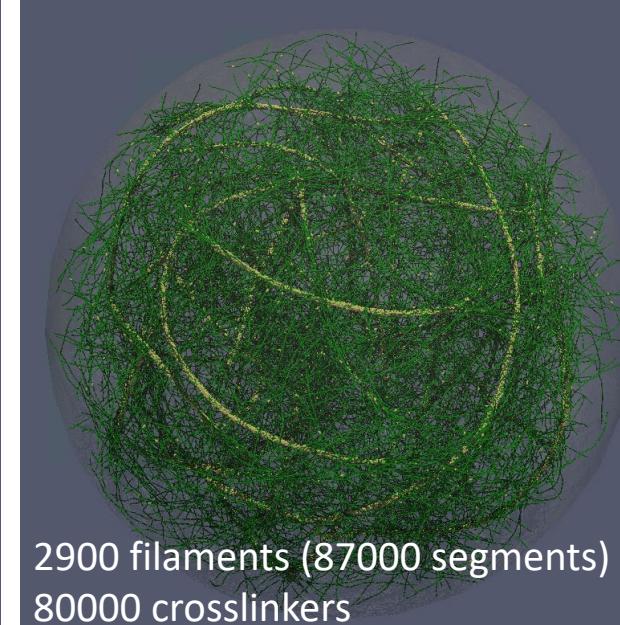
D = 8 μm



D = 12 μm

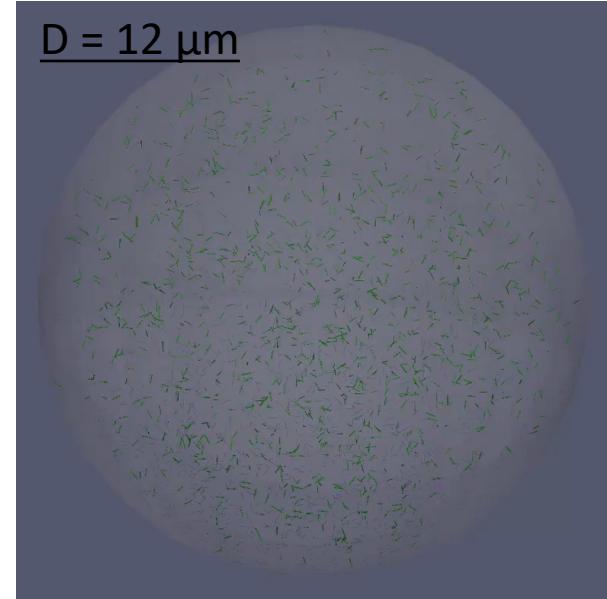
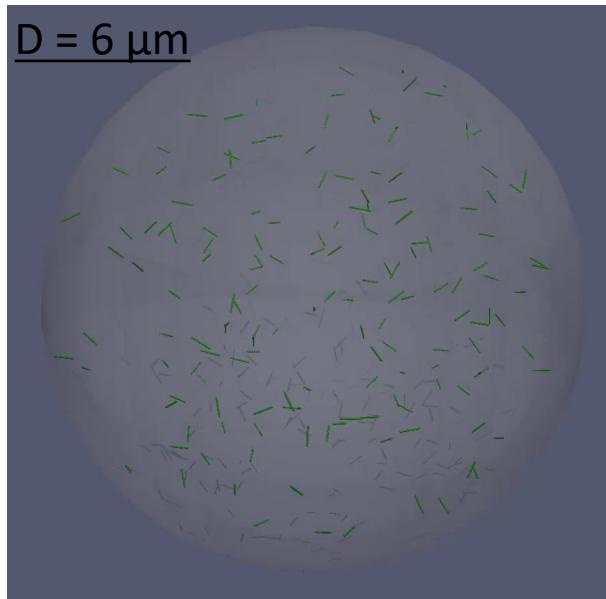


D = 16 μm



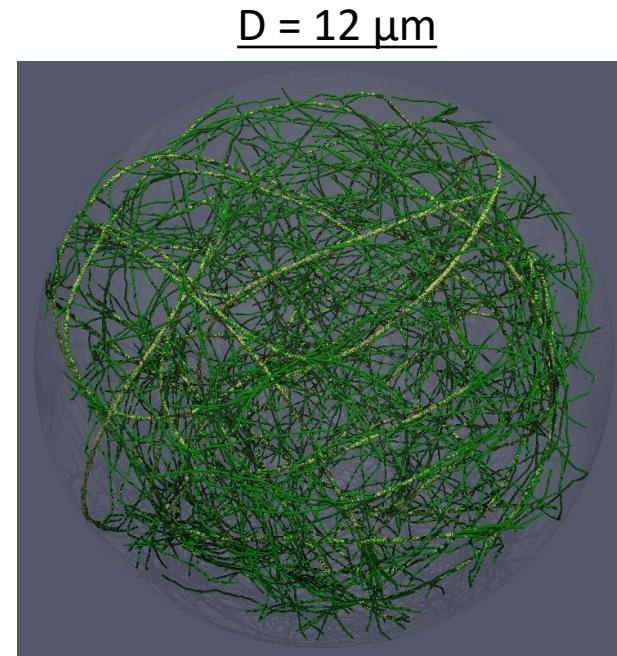
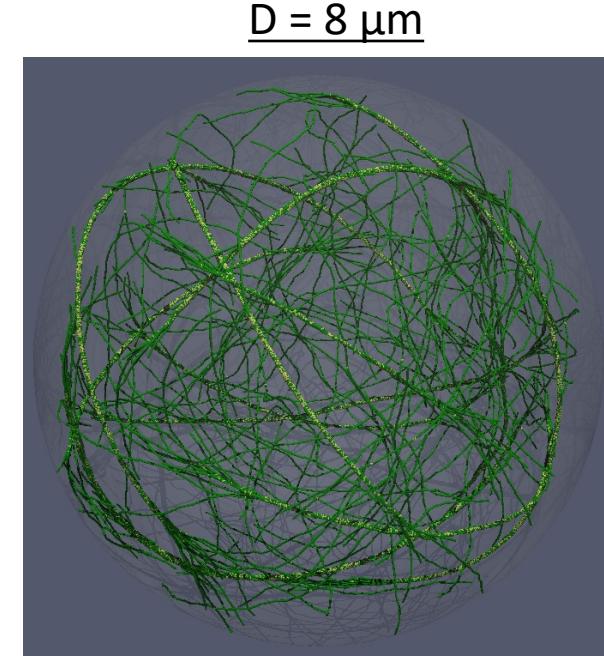
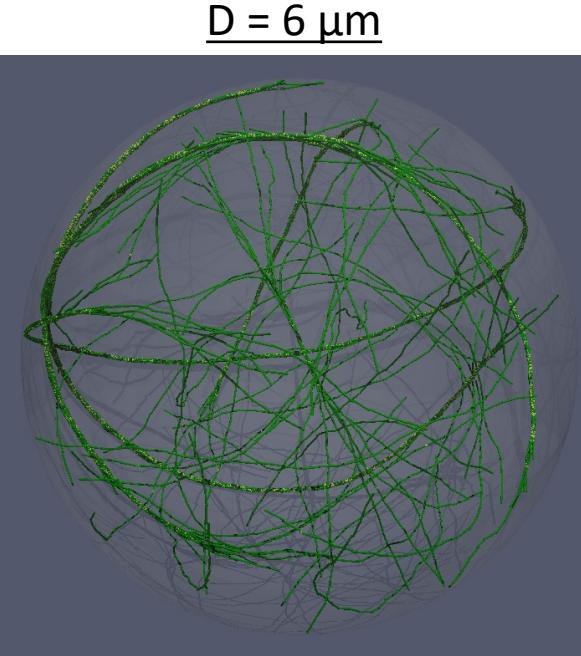
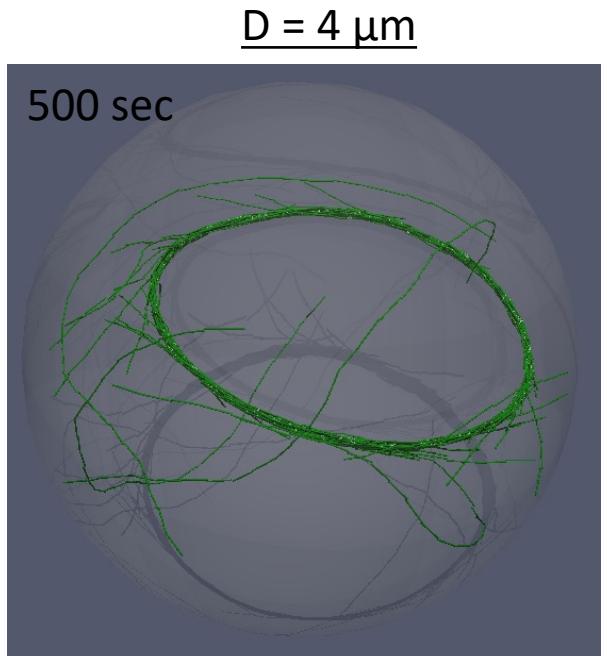
Time Lapse Movie of Simulation:

- Filament elongation simulated as addition of $0.2\text{ }\mu\text{m}$ segments at barbed ends (simultaneously at all filaments)
- Growth slows down with time to simulate monomer depletion.
- Filaments grow to $6\text{ }\mu\text{m}$ ($=30$ rod segments) over ~ 200 sec



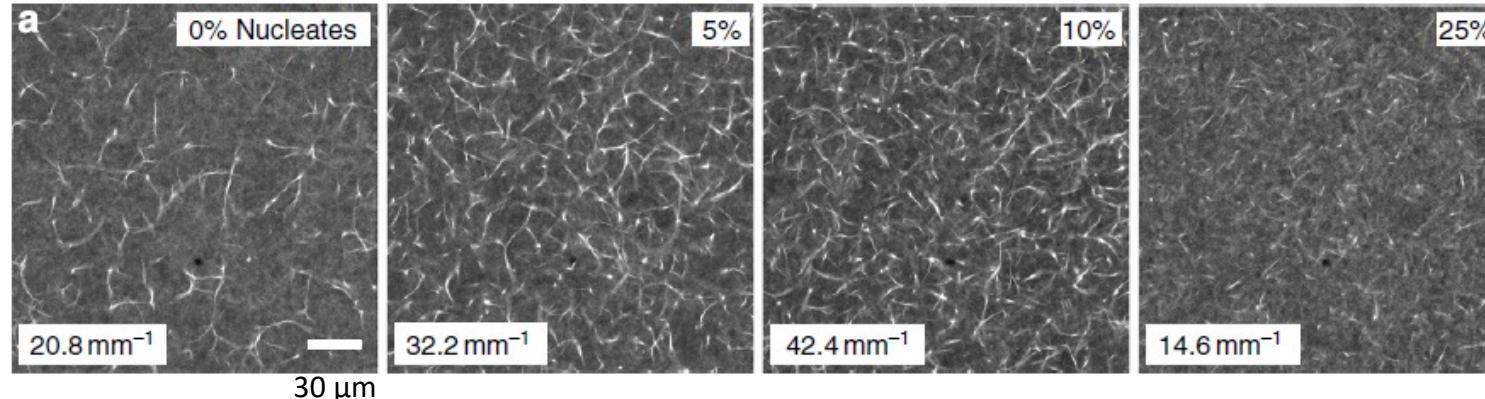
Oriented Rings Forming with Fascin

5 μM actin + fascin (short, parallel bundler)



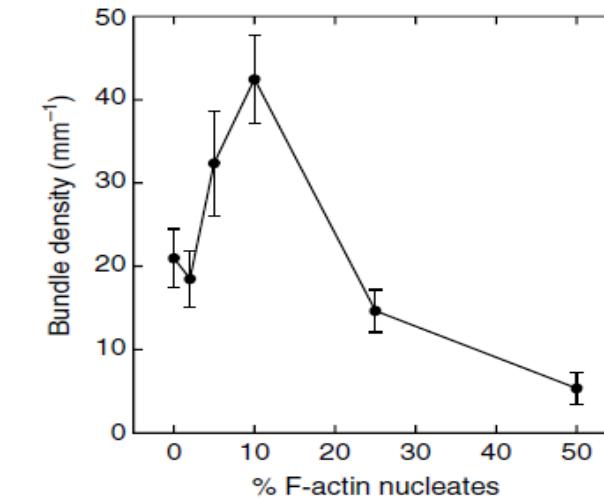
Simulating how bulk assembly kinetics determines structure of bulk cross-linked networks

Initial filament seed concentration determines final network structure

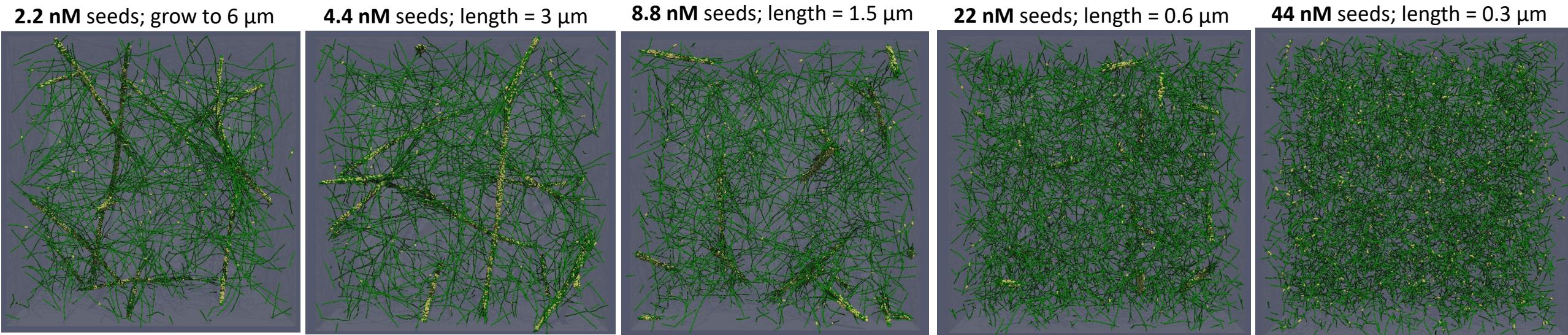


Falzone et al. Nat Comm 2012

5 μM actin, 1 μM α-actinin

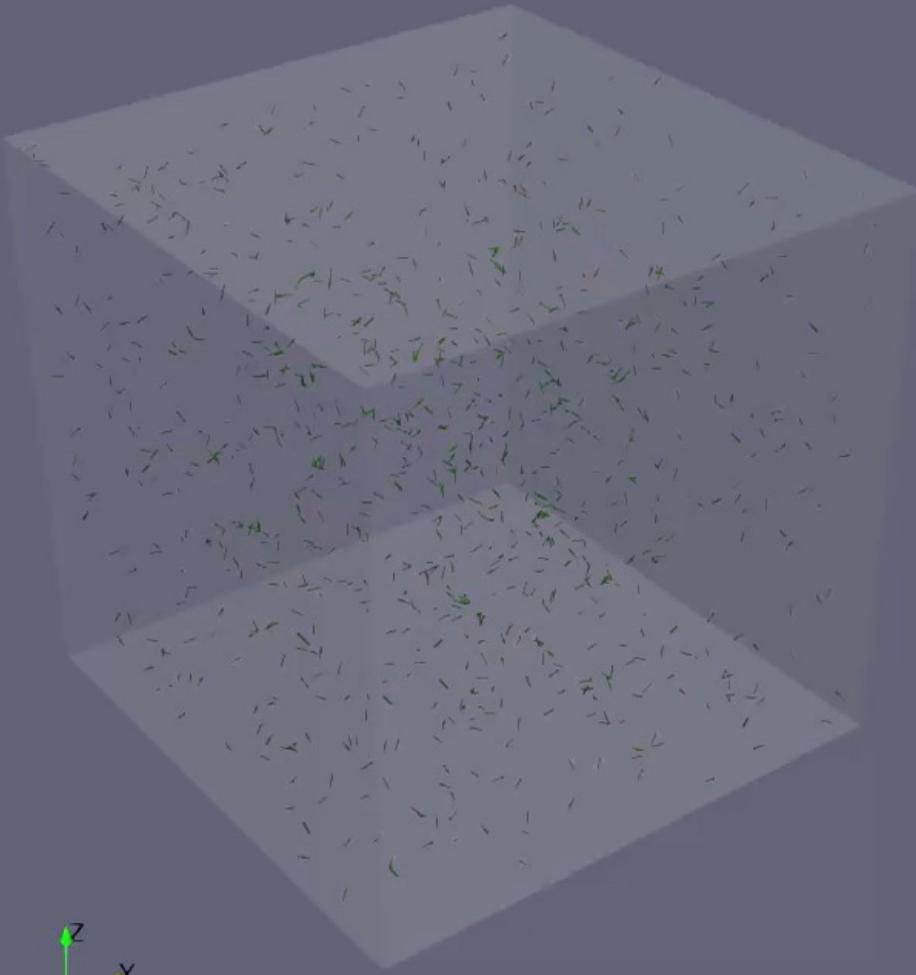


aLENS simulations, 5 μM actin, varying seed concentration

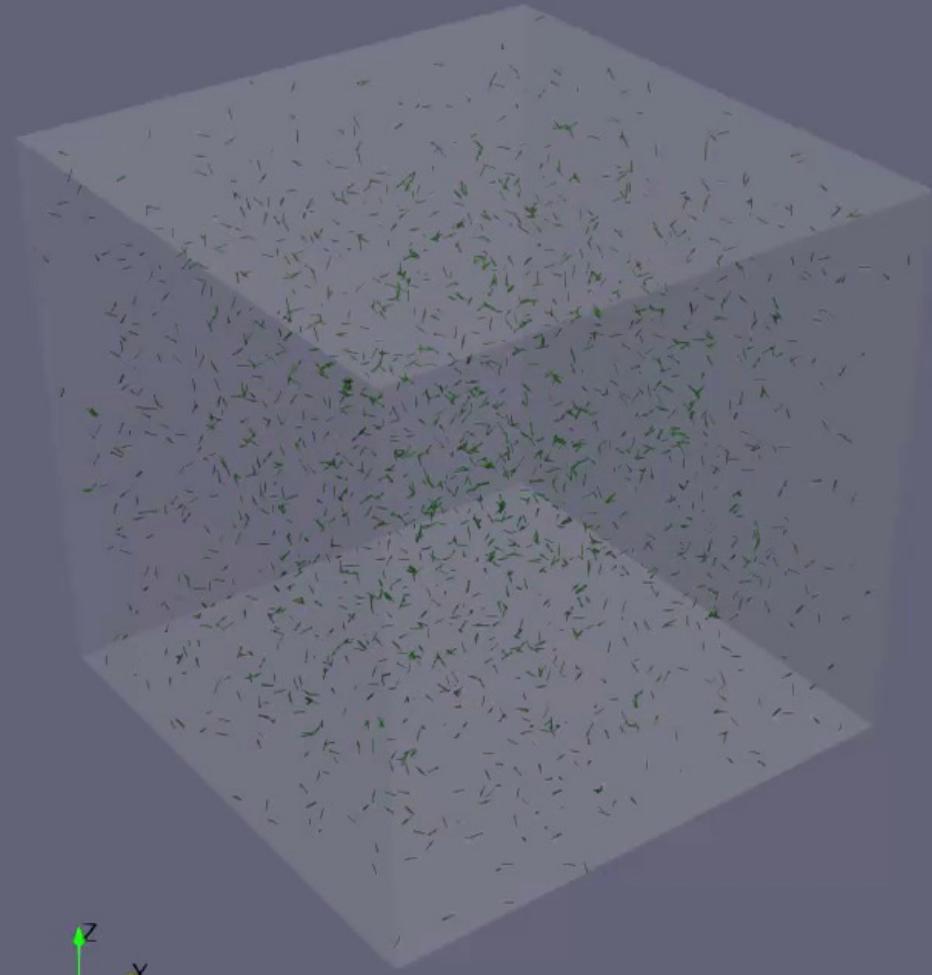


2.2 nM, final length = 6 μm

4.4 nM, final length = 3 μm



9 x 9 μm simulation box

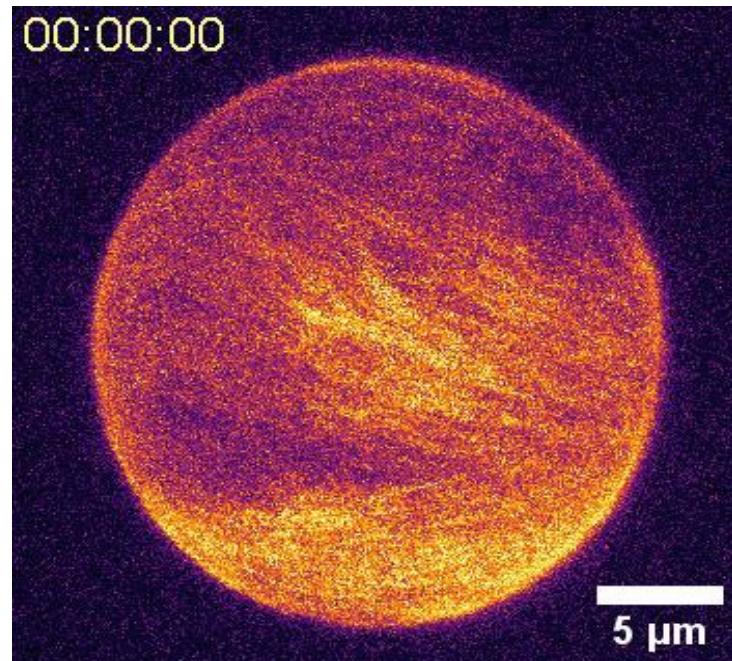


Total time shown: 130 s

Motor-driven patterns of confined actin filaments

Hsu et al. Nat Comm 2022

“polar band” state



$L = 0.6 \mu\text{m}$, HMM motors on inner leaflet, PEG depletant

- Prior models of driven filaments on flat and curved surfaces show a variety of patterns and associated +/- 1, 1/2 defects
- Prior models: continuum or quasi-2d approximations

Huber, et al. Science 2018

Henkes, Marchetti, Sknepnek PRE 2018

Moore et al. Soft Matter 2020

- Importance of movement in 3d and depletion interaction to confine filaments on surface not fully understood

aLENS simulations with surface-attached motors + filaments of fixed length

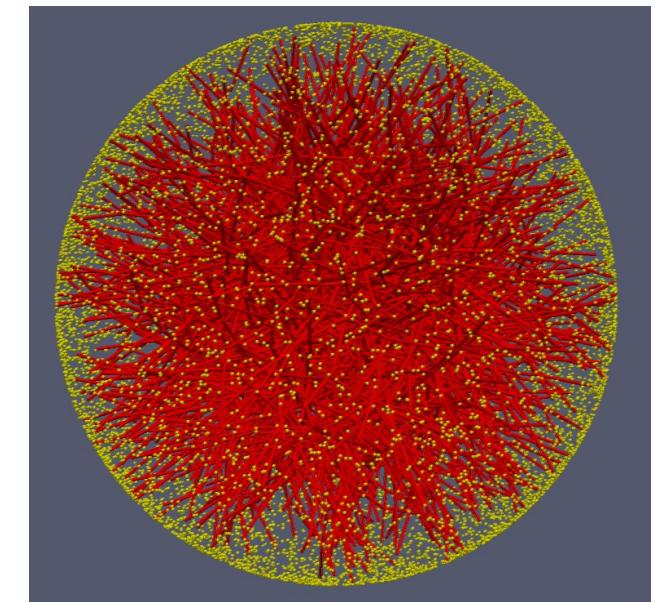


Yellow: Motors, 2 pN

$V_0 = 0.6 \mu\text{m/s}$

$F_{\text{stall}} = 2 \text{ pN}$, $k_{\text{off}} = 1 / \text{s}$

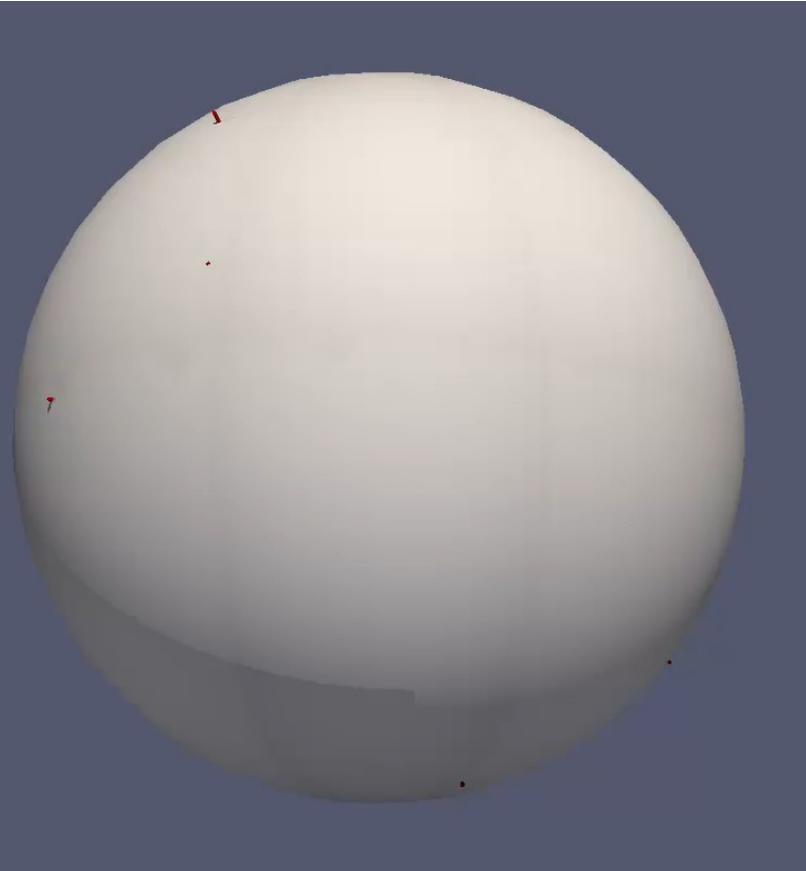
Sphere diameter = 2 μm



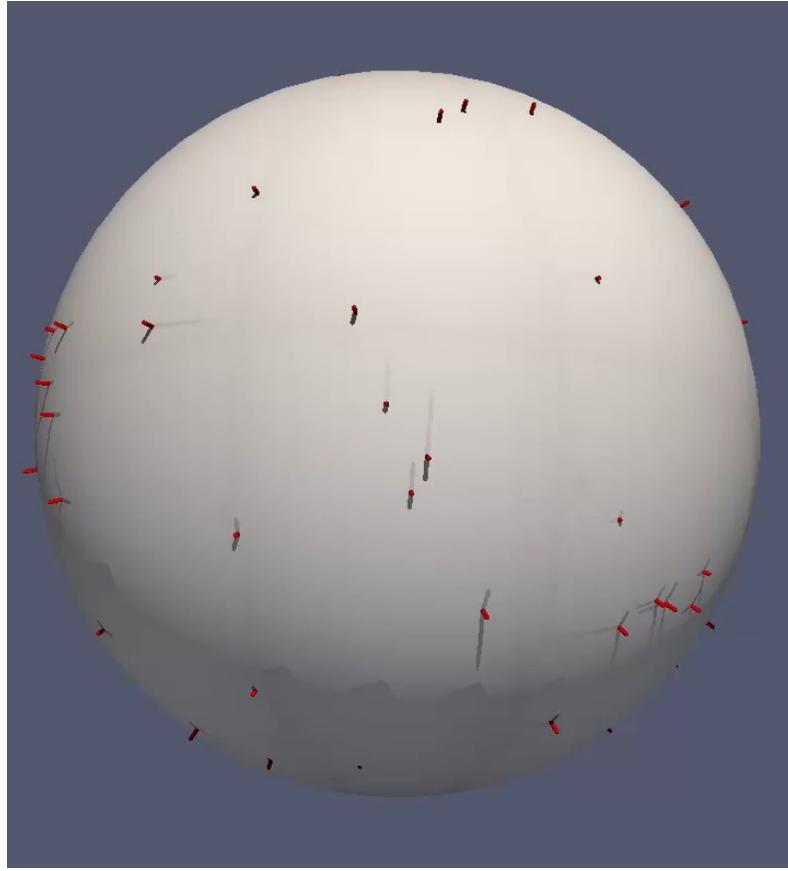
Nematic band state forms for large enough filament length

For display: sphere < confining diameter to highlight surface layer

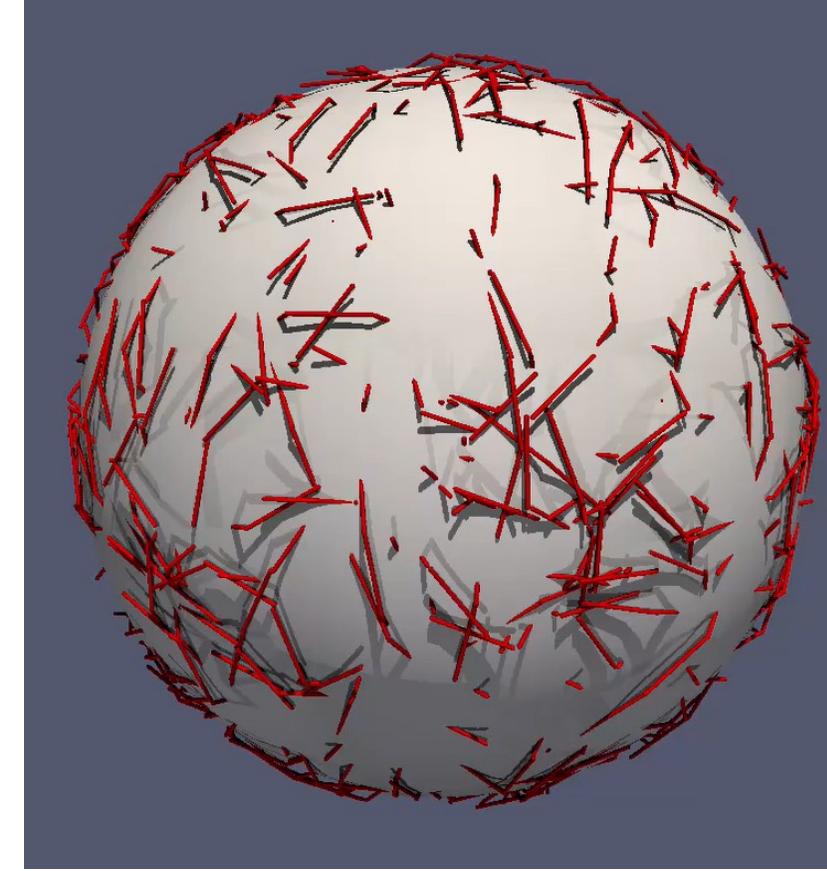
Length = 0.4 μm



Length = 0.8 μm



Length = 1.6 μm

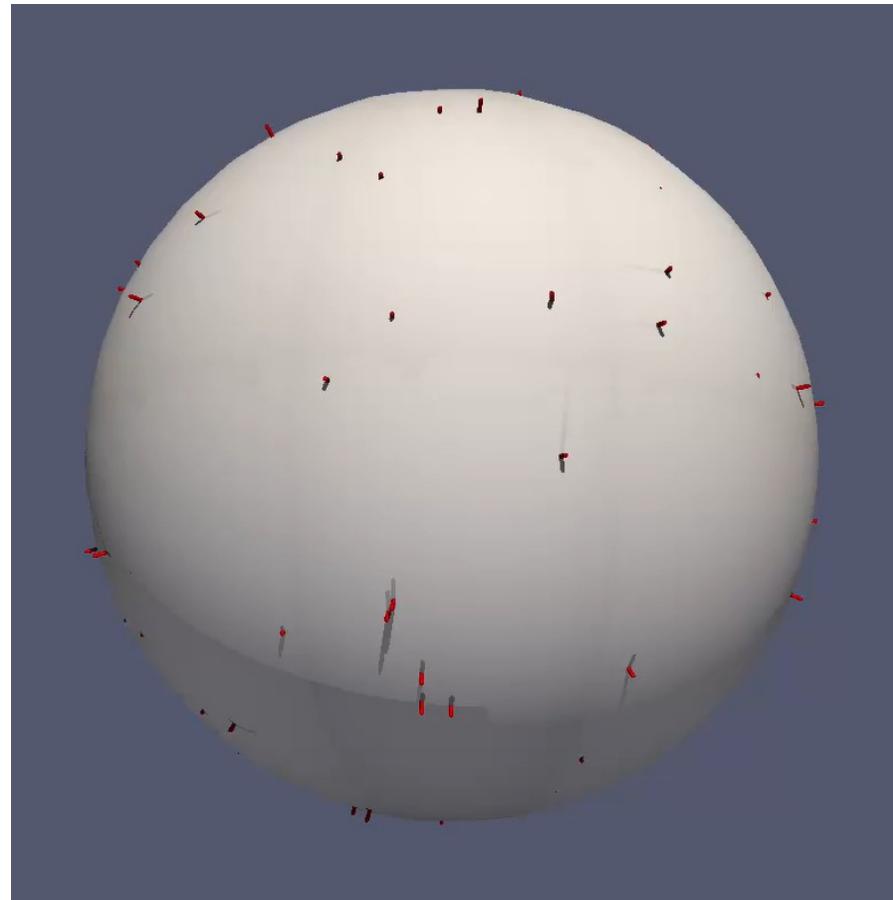


Confining sphere diameter = 2 μm

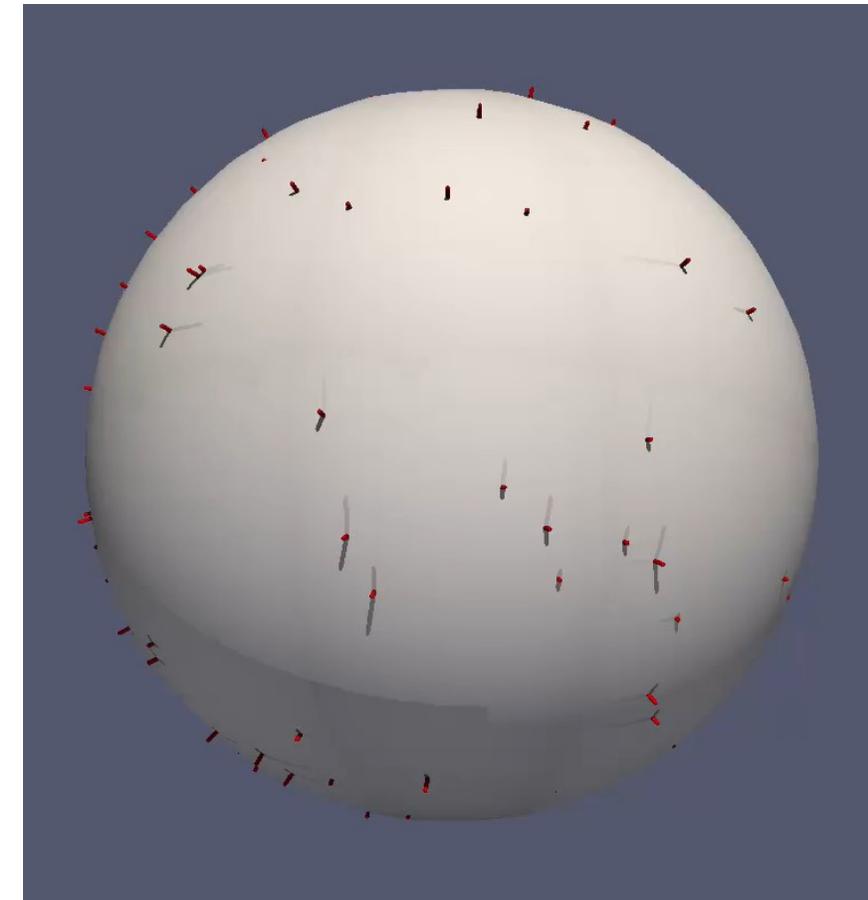
Introduction of α -actinin and fascin crosslinkers influences nematic order in opposite directions

For display: sphere < confining diameter to highlight surface layer

Length = 0.8 μm + α -actinin (yellow)



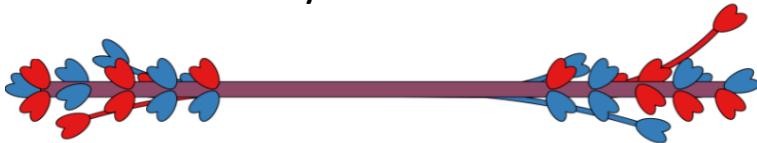
Length = 0.8 μm + fascin (yellow)



Confining sphere diameter = 2 μm

- Myosin II filaments with excluded volume can also be implemented

Non muscle myosin II filament



(Kaufman+Schwartz 2020)

Coarse-grained myosin II motors



8 coarse-grained motor heads/filament

Similar to T. Kim. Biom. Model. Mechano Biol. (2015)
accounting for tail excluded volume



David Rutkowski

