

Model of Yeast Actin Cable Distribution and Dynamics

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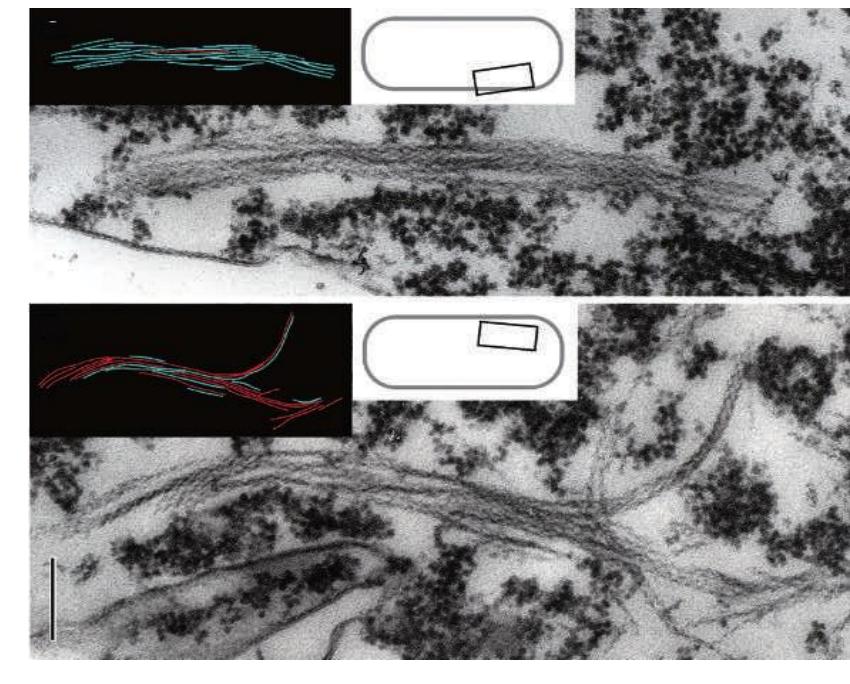
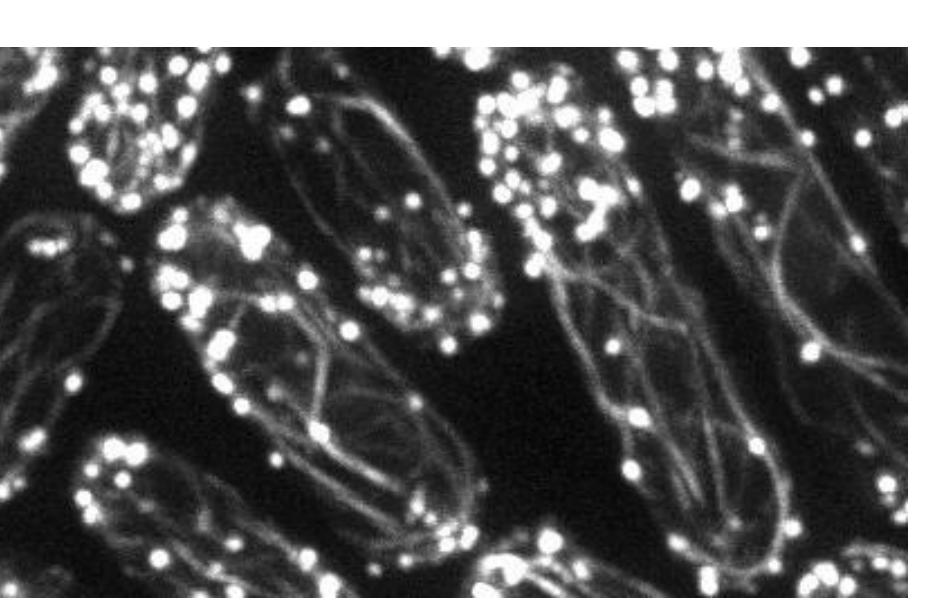
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Abstract

Formin For3p that localizes at the cell tip cortical sites nucleates actin to form actin filaments. These actin filaments bundle to form actin cables that span the cell and guide the movement of vesicles toward the cell tips. Since actin cables are structures whose dynamics can be monitored by fluorescence microscopy, and since yeast is a tractable genetic system, comparison of the results of theoretical models of actin cables to experiment could enable quantitative tests of the mechanisms of actin polymerization in cells. We used computer simulations to study the spatial and dynamical properties of actin cables. We simulated individual actin filaments as semiflexible polymers in 3D, composed of beads connected with springs. Formin polymerization was simulated as filament growth out of cortical sites located at cell tips. Actin filament severing by cofilin was simulated as filament turnover. We added attractive interactions between beads to simulate filament bundling by actin cross-linkers such as fimbrin. MyoV walking was simulated as tangential force along the cable. Comparison of the results of the model to prior experiments suggests that filament severing, nucleation, cross-linking and myosin walking are sufficient to describe the many features of actin cables. We were able to quantitatively analyze the bundled and unbundled phases as formin cluster density, cross-linking range and strength were varied. The simulated results of myoV-deletion yeasts are in agreement with the experiments.

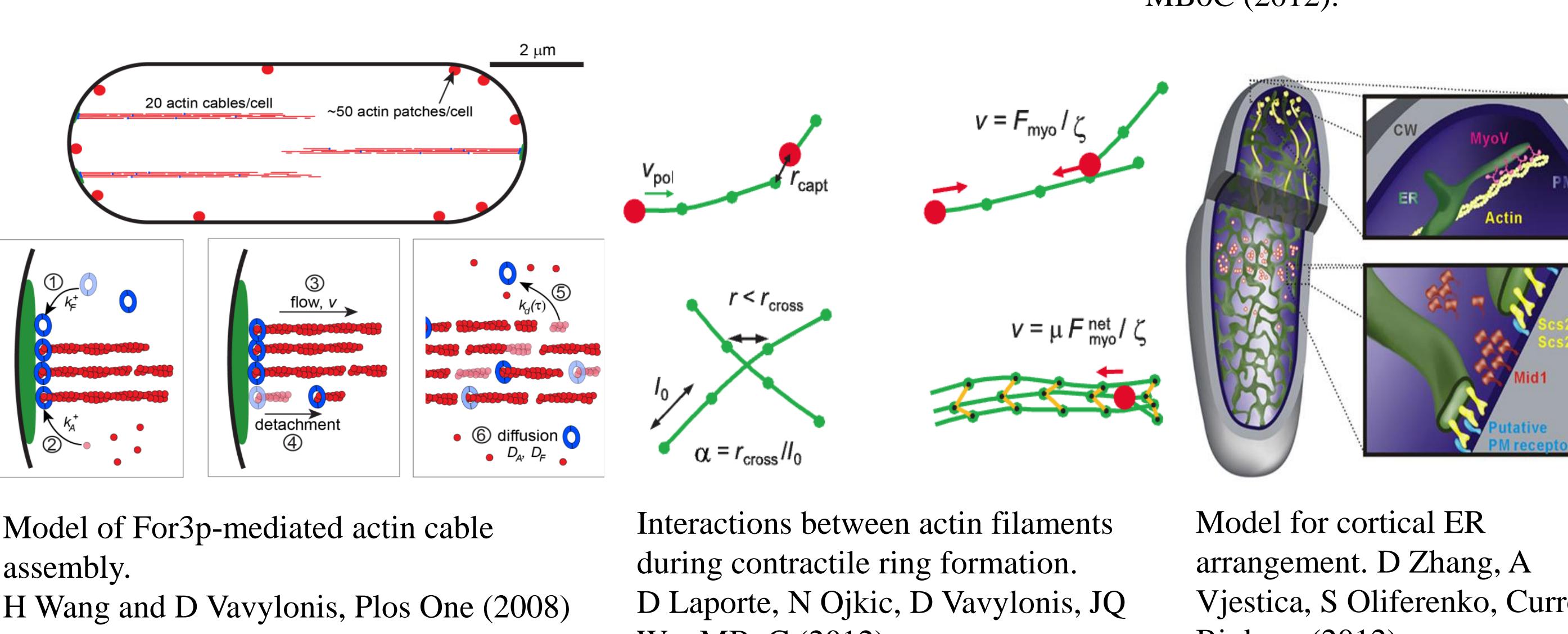
Introduction

- Formin For3p proteins on the cortical sites of the cell tip nucleate actin filaments that form bundles aligned along the axis of fission yeast.
- Actin cables are bundles of filaments aligned along the axis of fission yeast.



CHD-GFP labeled actin displaying actin cables and patches in fission yeast. Jian-Qiu Wu (OSU)

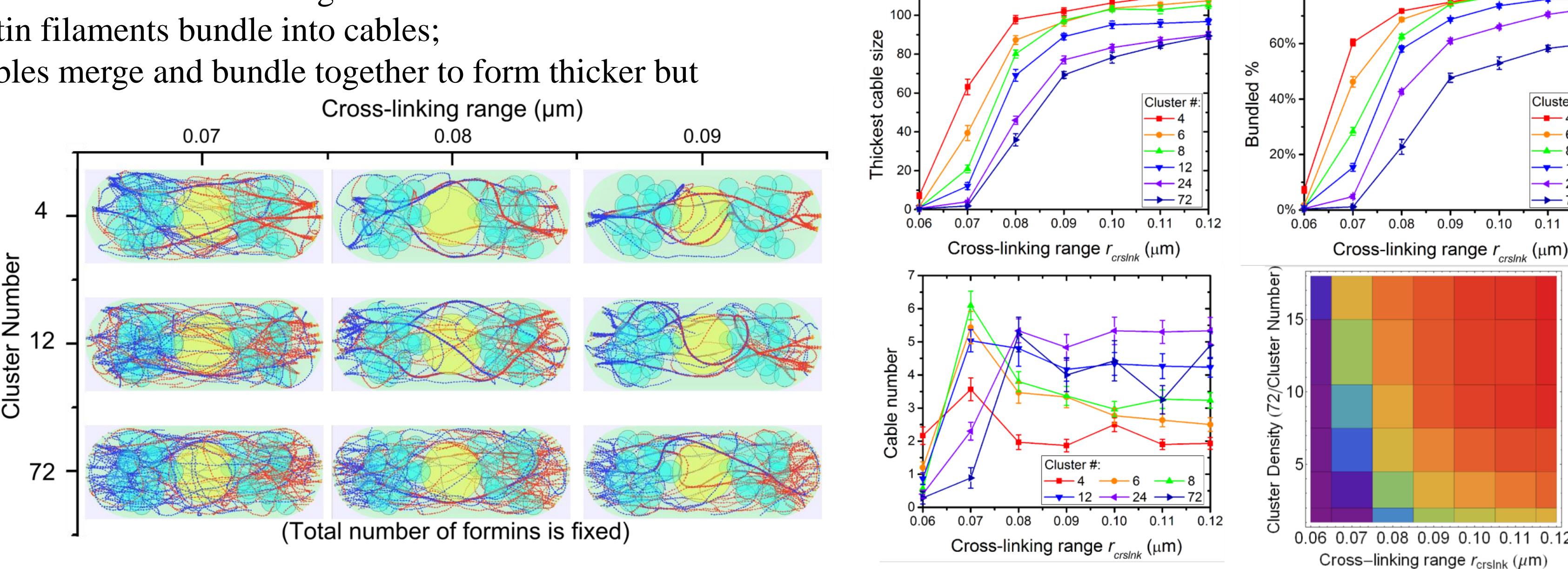
- Two actin filament cross-linkers in fission yeast, α -actinin (Ain1p) and fimbrin (Fim1p), can bundle actin filaments into parallel or antiparallel orientations. (C. Skau et al. JBC (2011), Laporte et al., MBoC (2012))
- Motor protein myosin V, carrying secretory vesicles or anchoring on organelles, walks along actin filament towards the barbed end.
- The ER is attached to the plasma membrane and to cables by myosin V. D Zhang, A Vjestica, S Oliferenko, Curr Biol (2012).
- For3p forms clusters at cell tips. Martin and Chang, Dev. Cell (2006), J. Dodgson et al. Nature Comm. (2013).
- For3p-mediated actin cable assembly and bundling of actin filaments have been previously modeled in fission yeast.



RESULTS

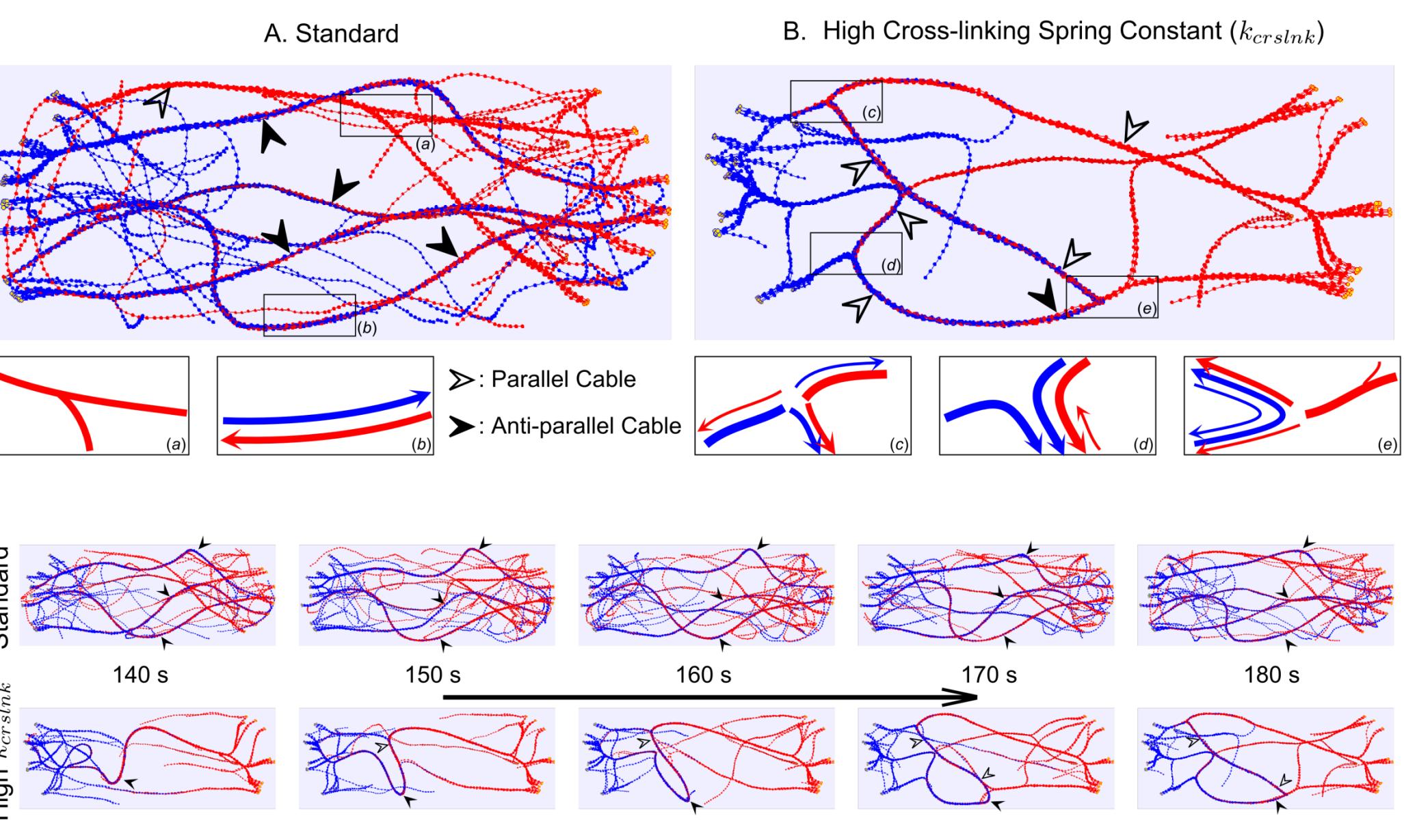
Both formin clustering and actin filament cross-linking affect cable formation

- Higher crosslinking (simulated by longer crosslinking interaction range r_{crslnk}) leads to thicker/fewer bundles.
- Denser formin clusters at the tip (smaller # clusters under fixed total filament number) enhance actin cable formation.
- Actin cable formation involves two stages:
 1. Individual actin filaments bundle into cables;
 2. Thin actin cables merge and bundle together to form thicker but fewer cables.



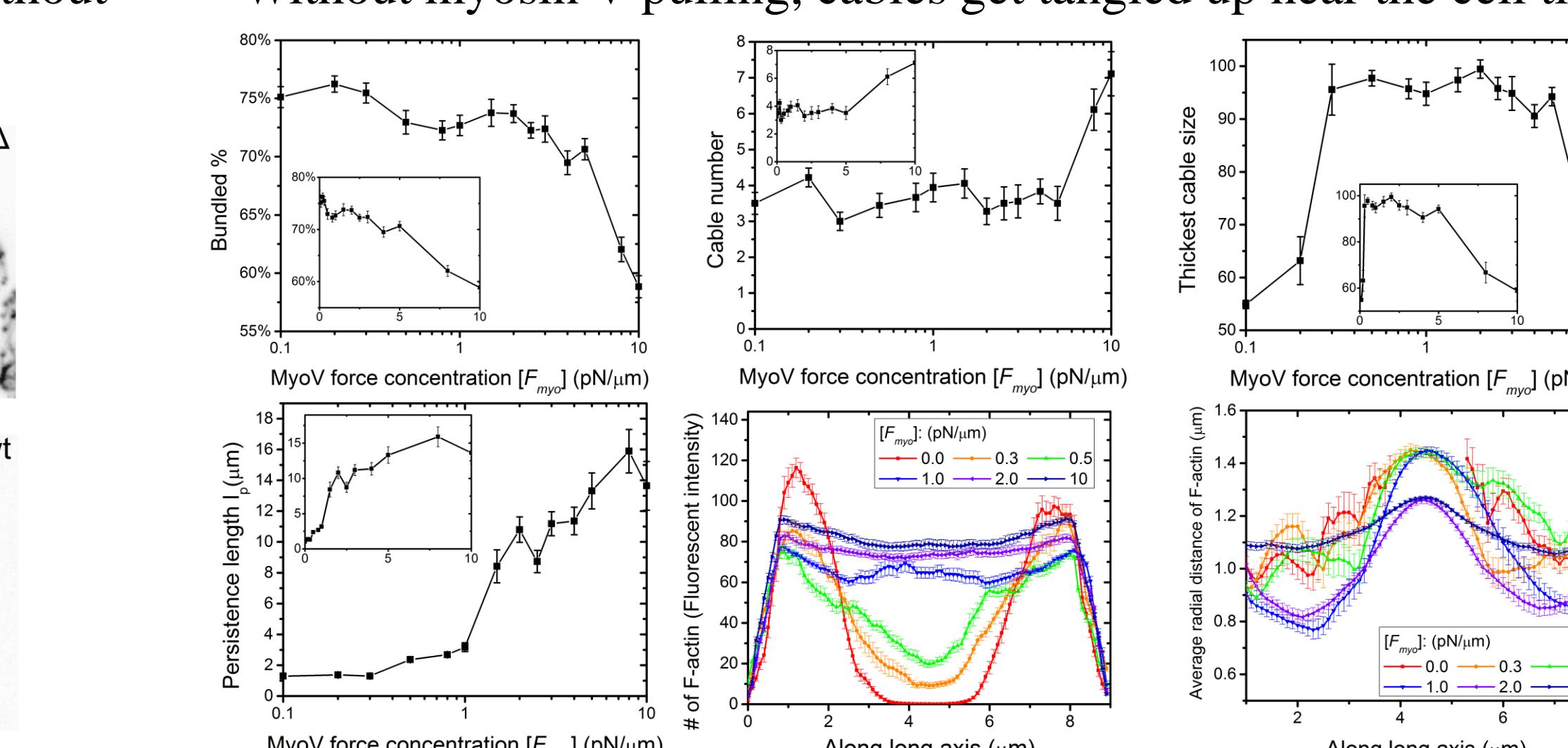
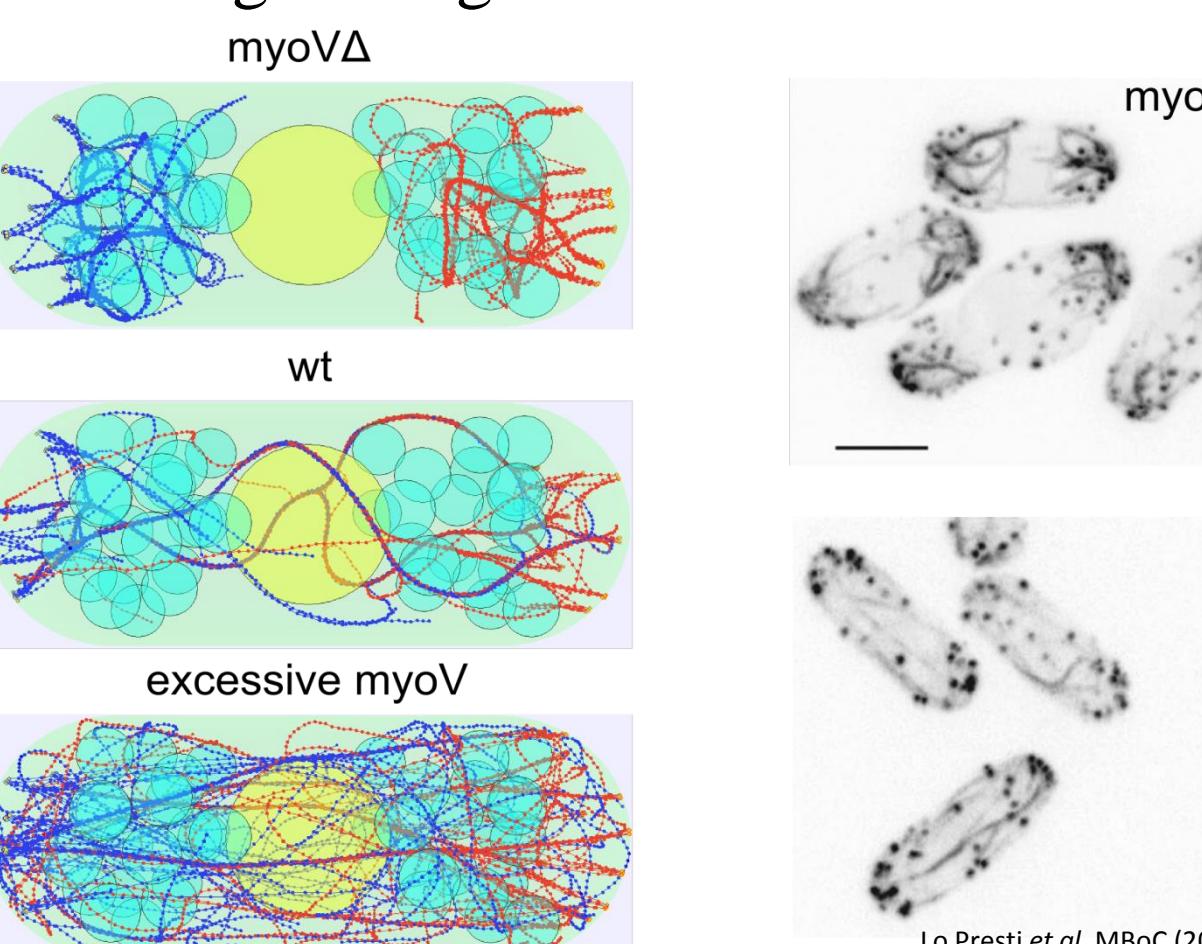
Crosslinking parameters affect cable distribution, dynamics and polarity

- Spring constant k_{crslnk} and range r_{crslnk} determine strength and lifetime of cross-linking.
- Dynamics include sliding, buckling and bulging.
- At high k_{crslnk} (permanent cross-linking), filaments tend to bundle in parallel; anti-parallel cables are unstable and will bulge to form parallel ones.



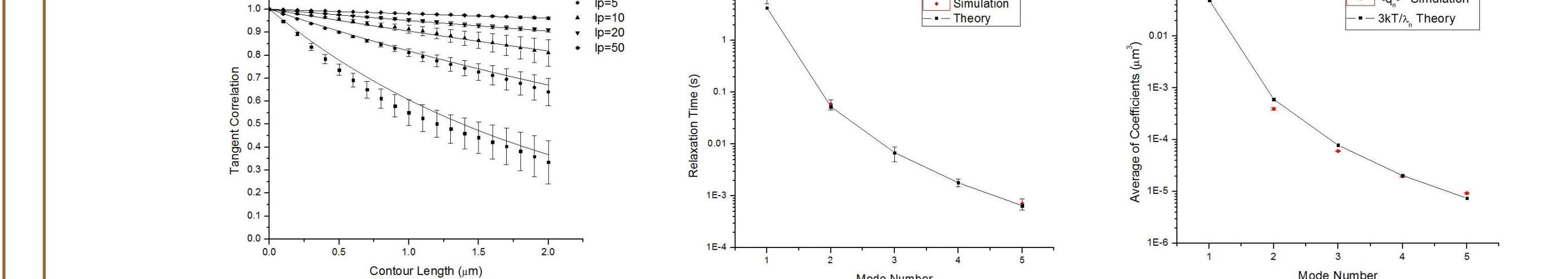
Myosin V pulling stretches actin cables

- In simulations myosin V straightens and stiffens the actin cables relative to simulations without myosin V.
- Simulated system compares favorably to observations of actin cables in wild-type and myoV Δ , where cells without myosin V exhibit straightening cable defect.



Model

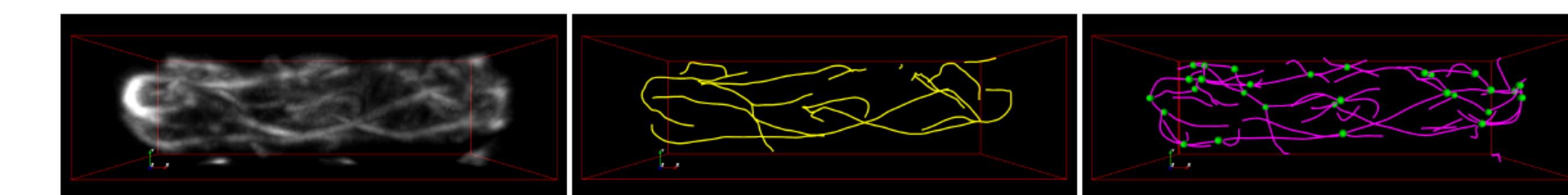
- Bead-spring model is used to simulate a single actin filament in 3D.
- Spring, bending and thermal forces together influence the dynamics of the actin filament.
- Short range attraction simulates the cross-linking proteins binding actin filaments to form cables or networks.
- Formin For3p polymerizes actin filaments at barbed end on the cortical sites of the tips.
- Fission yeast cell boundary is simulated as a fixed cylinder capped by hemispheres at both ends.
- Severing by cofilin simulated as filament turnover.
- Myosin V binding simulated as a transient tangential force towards the barbed end.
- Nucleus is simulated as an immobile sphere at the center of the cell.
- Vacuoles and organelles are approximated as immobile spheres randomly distributed inside the cell, limiting the volumes accessible to filaments.



Parameter	Description	Value	Parameter	Description	Value
N	Number of the formins at one tip (total $2N$)	72	τ_{sever}	Persistence length of the F-actin	10 μm
N_{cluster}	Number of clusters	12, 4-72	D_{yeast}	Filament model segment length	0.1 μm
D_{yeast}	Diameter of the yeast model	3.6 μm	k	Spring constant	100 pN/μm
L_{yeast}	Length of the yeast model	9 μm	η	Viscosity	0.301 pN·μm ² ·s
N_{vac}	Number of the vacuoles	40	D_{vac}	Cross-linking gap distance	2, 0-15 μm
D_{vac}	Diameter of the vacuoles	0.8 μm	D_{nuc}	Cross-linking interaction range	0.03 μm
D_{nuc}	Diameter of the nucleus	2.4 μm	r_{crslnk}	F_{myo} concentration = $F_{\text{myo}} \cdot [M_{\text{Myosin}}]$	0.09, 0-0.16 μm
v_{pol}	Polymerization rate (barbed end growth)	0.3 μm/s	t_{myo}	Myosin lifetime	0.5, 0-10 μm
τ_{sever}	Severing characteristic time by cofilin	15 s			5 s

Ongoing: compare to automated 3D segmentation results

- Multiple stretching open active contours: SOAX (Xu, Vavylonis, Huang, Medical Image Analysis (2013))



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Summary & Conclusions

- Simulated 3D distribution and dynamics of actin cables in fission yeast, including formin polymerization, cross-linking, turnover, myosin V pulling.
- Cross-linking generates bundles of actin filaments that resemble actin cables even when filament polymerization occurs randomly along cell tip.
- “Phase transition” of parallel and anti-parallel actin cable bundling by crosslinking r_{crslnk} and k_{crslnk} .
- Simulated myosin V walking stiffens the actin cables and prevents cables from tangling, in agreement with prior experiments.
- Myosin pulling force affects the cable distribution and radial distance at the cell center.