filaments to the membrane, is assumed to contribute a strongly attractive component to the potential. The polymerization and depolymerization rates depend on the filament-membrane gap. We model the elasticity of the actin network by linear springs connecting adjacent filaments to each other. The inner filaments of the array are taken to correspond to a Sla2 patch. They are thus bound more strongly to the membrane, and grow more slowly. The simulation results show that the outer filaments push on the membrane, while the inner filaments pull on it. We calculate the total pulling force as a function of several model parameters, including the potential depths, the free filament on- and off-rates, the numbers of fast- and slow-growing filaments, the filament tip stiffness, and the network rigidity.

[1] A. E. Carlsson and P. V. Bayly, Biophys. J. **106**:1596-1606(2014).

720-Pos Board B490

Formin's Processivity under Applied Force

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Formin, a key actin regulator, is involved in a number of pathologies. It is able to keep tracking the barbed end of actin filaments for a finite time, while accelerating actin polymerisation in the presence of profilin. In cells, formin's behaviour is precisely regulated by different factors, including local actin/profilin concentration, mechanical forces and other actin associated proteins. Clarifying the mechanism of formin's processive movement is essential in order to understand formin's behaviour. However, so far, how formin's processivity responds to various chemical or mechanical conditions is still unclear.

Here, we investigated how formin dissociates from the barbed end for different actin/profilin concentration and/or for various applied pulling forces, in order to answer 1) whether profilin has an impact on formin's processivity; 2) how formin's processivity responds to applied forces.

We found that formin's dissociation rate (k_{off}) from actin barbed end has a positive correlation with its elongation rate (V_{elong}) at fixed profilin concentration. However, when the concentration of profilin is increased, k_{off}/V_{elong} is decreased. These results indicate that formin's processive movement is improved by the presence of profilin.

Also, we found that formin's processivity is very sensitive to applied pulling forces. Formin's dissociation rate increases exponentially with force, even at relatively small force (<10 pN) is applied. Moreover, we found that force has a dominant impact on formin's processivity, for all actin/profilin concentration that we tested, irrespective of the elongation rate.

Finally, our study of formin's processivity will contribute to develop a more comprehensive model to describe formin's behaviour, especially how formin responds to mechanical forces in cells.

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MDIA1 Senses Both Force and Torque during F-actin Filament Polymerisation

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Formins play a crucial role in regulating actin polymerization. A formin dimer binds to the barbed end of F-actin through its ring-like dimerized FH2 domains and recruits G-actin monomers through its proline-rich FH1 domains in a profilin dependent manner. Formins are often subject to mechanical stretch due to actomyosin contraction; therefore its function has been believed to be dependent on mechanical force. We have recently investigated the effects of force on formin-dependent actin polymerization. In both the presence and the absence of profilin, we observed that force could strongly promote actin polymerization, which strictly required that the actin filament could freely rotate around the formin. These results revealed that formins senses not only tensile force but also torque in the filament. Together, these results provide important new insights into the mechanosensing functions of formin-dependent actin organization in living cells.

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Competition Among Multiple Pathways for Subunit Addition in Formin-Mediated Actin Filament Elongation

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The formin family of proteins nucleates and directs the growth of unbranched actin filaments. Formin-mediated elongation of actin filaments is accomplished via association of dimeric Formin Homology 2 (FH2) domains with filament barbed ends, where they enable subunit addition while remaining processively attached to the end. FH2-barbed end complexes exist in a rapid equilibrium between polymerization-competent and -incompetent states, so polymerization mediated by FH2 domains is relatively slow. This effect can be overcome by binding of actin monomers to profilin, which also binds polyproline tracts in formin's flexible FH1 domain, located directly N-terminal to the FH2 domain. Diffusion of FH1 domains brings associated profilin-actin complexes into contact with the FH2-bound barbed end, promoting direct transfer of actin and speeding elongation. As such, formins present multiple pathways for the addition of incoming monomers to an elongating barbed end. We investigated the relative contributions of FH1- and FH2-mediated pathways for elongation to the overall rate of polymerization promoted by Bni1p, a formin from *S. cerevisiae*. We found that the elongation rates mediated by the FH1 and FH2 domains are additive, suggesting that these domains contribute to elongation in an independent manner. In contrast, the four polyproline tracts in the FH1 domain of Bni1 promote elongation in a competitive manner. This competition is not position-specific, and thus does not result from steric clashes arising from binding of profilin-actin to neighboring binding sites. Rather, our results suggest that multiple FH1-bound profilin-actin complexes compete for delivery to the same binding site at the barbed end. This competition among polyproline tracts may limit the number of profilin binding sites in formin FH1 domains and may tie this number to FH2 domain gating, which controls the availability of the barbed end for binding.

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Multiscale Model of the Formin Homology 1 Domain Illustrates its Role in Regulation of Actin Polymerization

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Formins are important actin regulators. Formins bind profilin to polyproline tracks of its believed flexible Formin Homology (FH) 1 domain. The FH2 domains wrap around the barbed end of the actin filament and elongate the filament processively. Profilin-actin complexes on the FH1 domain are modeled to transfer to the barbed end; however, the mechanism is not known. Previous models of the FH1 domain have not captured sequence-specific effects such as the length and distribution of the polyproline tracks and possible variety in mechanosensitivity and response to bound profilin/profilin-actin. To remedy this, we perform simulations of the FH1 domain of well-studied formins: the mouse formins mDia1 and mDia2, the budding yeast formins—Bni1 and Bnr1, and the fission yeast formins—Cdc12, Fus1, and For3. We perform all-atom molecular dynamics simulations of each of these FH1 domains and show that FH1 is a typical intrinsically disordered protein (IDP), with the polyproline tracks forming high propensity poly-L-proline helices. We develop an alpha-carbon coarse-grained model that retains the sequence-specificity of the FH1 domain which is consistent with the IDP notion of FH1, and use this to study the FH1 domain in the context of its biological role. We use the coarse-grained model to investigate the response of FH1 to force and bound profilin. We show how bound profilin/profilin-actin may extend the FH1. We show definitively that multiple profilin-actin complexes can simultaneously bind to the FH1, which may be biologically important given the relatively high concentration of profilin-actin *in vivo*. We examine the transfer mechanism in further detail. We show how the FH1 may be affected by being on the FH2-bound actin filament barbed end rather than in isolation and examine the geometry of this system with bound profilin-actin.

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The *Drosophila* Formin FHOD Nucleates Actin Filaments

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Formins are a conserved group of proteins that nucleate and processively elongate actin filaments. Among them, the formin homology domain-containing protein (FHOD) family of formins contributes to contractility of striated muscle and cell motility in several contexts. However, the mechanisms by which they carry out these functions remain poorly understood. Mammalian FHOD proteins were reported not to accelerate actin assembly *in vitro*, and they have instead been suggested to act as barbed end cappers or bundlers. Here, we show that purified *Drosophila* Fhod and human FHOD1 both accelerate actin assembly by nucleation. FHOD1's nucleation activity is restricted to

cytoplasmic actin, whereas *Drosophila* Fhod potently nucleates both cytoplasmic and sarcomeric actin isoforms. We found that *Drosophila* Fhod binds tightly to barbed ends, where it slows elongation in the absence of profilin and allows elongation in the presence of profilin. Fhod protects barbed ends from capping protein, but dissociates from barbed ends relatively quickly. Finally, we used cosedimentation assays to determine that Fhod binds the sides of actin filaments and bundles filaments. This work establishes that Fhod shares the capacity of other formins to nucleate and bundle actin filaments, but is notably less effective at processively elongating barbed ends.

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Cofilin Induces a Local Change in the Twist of Actin Filaments

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Cofilin is a small protein that binds and severs actin filaments near the boundaries between cofilin-decorated and bare actin segments. Cofilin binds cooperatively to actin by inducing conformational changes that propagate along the helical lattice to neighboring bare actin segments, favoring cofilin binding. However, the extent to which these cooperative conformational changes propagate remains unclear and prior estimates for the propagation length vary widely; it has been suggested that anywhere from 2 to 24 actin subunits extending into the bare region could be affected. We address this unresolved question by imaging partially cofilin-decorated actin filaments using cryo-electron microscopy and determining the location of boundaries between decorated and undecorated regions at the subunit level using a novel particle subtraction and 3D classification strategy. We used characteristic changes in filament twist induced by cofilin as a marker for cooperative changes in actin, and tracked these changes with respect to the boundary. Our results indicate that cofilin induces a change in twist that propagates at most 2 subunits away from the boundary, irrespective of the polarity of the boundary (i.e. whether the bare side of the boundary extends toward the pointed or barbed end of the actin filament). These observations provide direct experimental support for nearest-neighbor models describing cofilin-binding cooperativity, and inform mechanistic models for cofilin-mediated actin severing.

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Mechanotransmission and Mechanosensing of Human Alpha-Actinin 1

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Alpha-actinins play crucial roles in organizing the framework of the cytoskeleton through crosslinking the actin filaments. However, the molecular mechanisms underlying its functions are still incompletely understood. In this work, by mechanical manipulation of single human alpha-actinin 1 using magnetic tweezers, we determined the mechanical stability and kinetics of the functional domains in alpha-actinin 1 as well as the mechanical strength of the alpha-actinin 1 dimerization interaction. Moreover, we identified the force-dependence of vinculin binding to alpha-actinin 1, with the demonstration that force is required to expose the high-affinity binding site for vinculin binding. Based on the mechanical stability and kinetics of alpha-actinin 1, a novel role of the alpha-actinin 1 as molecular shock absorber for the cytoskeleton network is revealed. Our results provide the first comprehensive analysis of the force dependent stability and interactions of alpha-actinin 1, which sheds new light on the molecular mechanisms underlying its mechanotransmission and mechanosensing functions.

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Computational Modeling of Ena/VASP Interacting with Actin Filament to Understand its Processivity

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Ena/VASP proteins enhance formation of filopodia in the cell membrane, which is composed of long and straight actin filaments. These proteins processively bind to the barbed end of actin filaments and increase actin elongation rate by two to three times. In this work, we first built an all-atom model of Ena/VASP interacting with actin filament (F-actin) by using computational protein design techniques since a complete structure of Ena/VASP interacting with F-actin is currently not available. We assessed the correct binding site of F-actin binding domain (FAB) of Ena/VASP on the F-actin by using well-tempered metadynamics (WTMetaD) simulations. The WTMetaD simulations demonstrated that FAB domain favors to interact with the cleft between subdomain (SD) 1 and SD 3 of the actin subunits, which is in a good agreement with a previous hypothesis. To understand the factors affecting the processivity of Ena/VASP at the barbed end of crosslinked filaments, we generated coarse-grained (CG) model of this structure by using a bottom-up approach, and then elaborated this CG structure to include four arms and two actin filaments connected with a cross-linker. We varied the number of arms in the CG model and investigated differences between their behaviors to understand the underlying mechanism of decreasing processivity with reduced number of arms as observed in the experiments. Our results showed that Ena/VASP becomes less stable on the filament when the number of arms decrease, which might be one of the factors that causes reduced processivity of Ena/VASP. The findings of this work can be used to resolve the underlying molecular mechanisms of actin network assembly/disassembly interacting with Ena/VASP.

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Binding of the N2A Region of Titin to Actin Filaments

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The sliding filament model has been the foundation of our understanding of muscle contraction, explaining contraction as the calcium-dependent formation of cross-bridges between actin and myosin filaments. While this has been the prevailing model for muscle contraction for over 50 years, a weakness of model is its inability to account for all the measurable forces observed in muscle contraction. Recently, it has been suggested that interactions between the titin filament and actin could be the missing component in existing models. The N2A region of titin sits in a unique region of titin, between the Ig-domain region that is elongated under low forces and the PEVK region, which is extended under higher forces. In addition, the muscular dystrophy with myositis (*mdm*) mice contain a deletion at the junction between the N2A and PEVK regions and muscles from these mice exhibit altered contractile properties. Based on these observations, we hypothesized that the N2A region might be a site of interaction between titin and actin and have used actin co-sedimentation and actin motility assays to investigate this possibility. We have demonstrated that recombinantly-expressed N2A constructs co-sediment with filamentous actin, suggesting an association between N2A and actin. In addition, *in vitro* motility data demonstrates reduced actin filament velocity in the presence of N2A, further supporting a binding interaction between N2A and actin. This work demonstrates that binding between actin and titin occurs *in vitro* and future work will be focused on demonstrating that this interaction also occurs in vivo to elucidate the role of this interaction in muscle contraction.

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Stochastic Simulations of Tropomyosin Binding and Diffusion on Filamentous Actin

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The actin-binding protein tropomyosin plays a key role in regulating both the interaction of filamentous actin (F-actin) with other binding proteins, as well as the bending rigidity and biochemistry of F-actin itself. Tropomyosin binds to F-actin in a 1:7 ratio, with one tropomyosin unit covering 7 actin monomers. Tropomyosin units can form end-to-end bonds with their neighbors, and have been experimentally observed to nucleate and form tropomyosin chains on the actin filament, which can elongate and decorate the actin filament. However, since tropomyosin spans 7 actin monomers, two such tropomyosin chains on the same actin filament have a 6-in-7 chance of being out of register, leaving a gap in decoration of one or more actin monomers where they meet.
Lateral diffusion of tropomyosin chains on F-actin was recently proposed as a potential mechanism by which gaps in decoration could be resolved. Here, we present stochastic computer simulations of tropomyosin binding to F-actin. We develop a theoretical framework based on the hydrodynamic drag experienced by tropomyosin during diffusion, and simulate tropomyosin's decoration to F-actin and the development and resolution of gaps in decoration over time. Our results support lateral tropomyosin diffusion on F-actin as a potentially essentially mechanism by which tropomyosin molecules can decorate filamentous actin without leaving gaps in decoration.

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C1 IG-Domain of Myosin Binding Protein-C Activates Cardiac Thin Filament by Means of Thethering Tropomyosin to the Subdomain-1 of Actin

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