The dynamic assembly and disassembly of filamentous actin (F-actin)\(^1\) and its organization in ordered arrays are coordinated by various accessory proteins (1). More than 60 actin-binding proteins have already been identified in mammalian cells, among which 7 are F-actin cross-linking/bundling proteins (2). The function of actin cross-linking/bundling proteins is believed to mediate interactions between actin filaments to form both orthogonal networks and ordered bundles. Orthogonal networks and bundles appear, sometimes concurrently, in specialized subcellular complexes, including lamellipodia, filopodia, stress fibers, and focal adhesions. These organelles, which coordinate cell migration and cell spreading (3), often contain more than one actin cross-linking protein. For instance, stress fibers in adherent epithelial cells and fibroblasts contain α-actinin, fascin, and tenasin (4), which are proteins shown to have the actin cross-linking/bundling function in vitro. Focal adhesions, which mechanically couple the actin cytoskeleton to the extracellular matrix via cell surface receptors integrins, contain the actin cross-linking proteins α-actinin, talin, plectin, filmin, and vinculin (5). Actin-filament bundles in stable microspikes and isotropic meshworks in the lamellipodia of motile cells also contain several actin cross-linking proteins, including fascin and α-actinin and the Arp2/3 complex, α-actinin, myosin, and fascin, respectively (6).

The fact that several actin cross-linking/bundling proteins co-localize to subcellular organelles suggests that these proteins complement each other and/or have overlapping functions. The relative tolerance of cells to null mutations of genes that code for a single cross-linking protein suggests that the functions of actin cross-linking proteins are highly redundant (7–9). This apparent functional redundancy may, however, reflect the limited resolution of available assays in assessing the functions of actin cross-linking proteins. The relative tolerance of cells to null mutations of genes that code for a single cross-linking protein suggests that the functions of actin cross-linking proteins are highly redundant (7–9). This apparent functional redundancy may, however, reflect the limited resolution of available assays in assessing the mechanical role of F-actin cross-linking/bundling proteins in vitro and in vivo.

Recent ultrastructural work on developing Drosophila has begun to elucidate the mechanisms by which actin cross-linking proteins that co-localize in certain subcellular regions could synergistically operate. Tilney and co-workers showed that singed and forked proteins, two putative actin cross-linking proteins, were both required for proper formation of actin filament bundles in the mechanosensitive bristles of Drosophila (10–12). Forked proteins are necessary in the early development of the F-actin bundles that singed proteins subsequently stabilize via a zippering mechanism. How singed and forked proteins can complement one another to produce the propulsive forces required for the development of sensory bristles is however unknown.

Networks of actin filaments exhibit relatively poor mechanical resilience, they only resist relative shear deformations of amplitude smaller than 3% (13); moreover, the elasticity of F-actin networks in vitro is much lower than that of that found in living cells (14). Actin filaments, therefore, harness auxiliary proteins to self-organize into stiff structures that can strengthen the cytoplasm. Here, using reconstituted actin filament networks, we investigated how two types of actin cross-linking proteins could enhance the resilience of actin filaments synergistically. Resilience is measured by the shear amplitude at which the modulus of a network under stress begins to decrease, presumably due to the breakage and/or alignment of the filaments by the applied stresses. This work focuses on
**Synergy of F-actin Cross-linking Proteins**

**Fig. 1.** Strain-induced softening of F-actin/fascin solutions. 

- **a** and **c**, time-dependent relaxation modulus, \( G(t, \gamma_0) \), of F-actin/fascin solutions containing 0.48 \( \mu \)M (a) and 1.2 \( \mu \)M fascin (c). Each F-actin/fascin solution is subject to a step deformation of preset amplitude \( \gamma_0 \) (indicated in the figure), a deformation that is maintained during the measurement of the relaxation modulus. The deformation creates a shear stress, which is dissipated by the movements of the molecules. The relaxation modulus would decrease rapidly if small molecules (e.g., water) were sheared and slowly for permanently cross-linked filaments. Relaxation moduli were obtained by dividing the measured time-dependent stress induced by the applied step deformation of increasing amplitude \( \gamma_0 \). 

- **b** and **d**, modulus of F-actin/fascin solutions as a function of the deformation amplitude \( \gamma_0 \) for fascin concentrations of 0.48 \( \mu \)M (b) and 1.2 \( \mu \)M (d). Inset, strain-dependent moduli of an F-actin solution in the absence of cross-linking proteins. Symbols in b and d correspond to the time scales 0.05 s (closed squares), 0.1 s (closed circles), 1 s (open squares), 10 s (open circles), and 100 s (closed triangles). Actin concentration is 24 \( \mu \)M in all experiments.

\[ \alpha \text{-} actinin \text{ and fascin, two F-actin cross-linking proteins that co-localize along stress fibers and in lamellipodia of epithelial cells and fibroblasts (15). Our rheological measurements show that these two proteins can generate microfilament arrays that are more resilient and more elastic than each one of these proteins separately. A finite element model of networks containing filaments and bundles show that a mechanical optimum is reached between an all-filament and all-bundle networks.} \]

**MATERIALS AND METHODS**

**Protein Purification**—Unless specified, all reagents were purchased from Sigma. Actin was prepared from chicken breast (16) using Sephacyr S-300 for gel filtration (17). Purified actin was stored as Ca\(^{2+}\)-actin in continuous dialysis at 4 °C against buffer G (0.2 mM ATP, 0.5 mM DTT, 0.2 mM CaCl\(_2\), 1 mM sodium azide, and 2 mM Tris-HCl, pH 8.0). Mg\(^{2+}\)-actin filaments were generated by adding 0.1 volume of 10× KMEI (500 mM KCl, 10 mM MgCl\(_2\), 10 mM EGTA, 100 mM imidazole, pH 7.0) polymerizing salt buffer solution to 0.9 volume of G-actin in buffer G. \( \alpha \)-Actinin was purified from chicken smooth muscle as described (18); \( \alpha \)-actinin was stored by dialysis against buffer G, which was changed daily with fresh buffer. Actin and \( \alpha \)-actinin were used within 5 days after purification. Human fascin was expressed as a glutathione S-transferase fusion using pGEX2T in the Escherichia coli strain JR600 as described in Ref. 19. Fascin was liberated from glutathione S-transferase by cleavage with thrombin, followed by glutathione-Sepharose chromatography. Fascin was dialyzed against 10 mM Tris, 1 mM DTT, pH 8.0, and applied to a DE52 column that was developed with a linear gradient of 0–70 mM NaCl in 10 mM Tris, 1 mM DTT, pH 8.0. Purified fascin was dialyzed against 10 mM Tris, 10 mM NaCl, 30 mM KCl, 0.1 mM EDTA, 1 mM DTT, pH 8.0, and stored at -70 °C.

**Mechanical Rheometry**—To probe the mechanical properties of F-actin solutions, we employed a strain-controlled mechanical rheometer (ARES-100 Rheotaxies, Paasutaway, NJ) as described (13, 20). The superior sensitivity of this rheometer is particularly well suited to probe low viscosity specimen such as F-actin networks. The G-actin solution was first mixed with either fascin, \( \alpha \)-actinin, or a mixture of fascin and \( \alpha \)-actinin in a test tube; 10× KMEI was added to the solution (total volume 1.4 ml). We note that we did not mix one of the cross-linkers with F-actin and then added the other because strong binding to F-actin can cause the early formation of heterogeneous structures. The solution was immediately loaded onto the lower plate of the rheometer. This plate is coupled to a computer-controlled motor, which can apply steady or oscillatory shear deformations of controlled frequency and amplitude (13). The upper tool of the rheometer is a truncated cone (0.04 radian), which is connected to a sensitive torque transducer that measures the stress induced within the F-actin specimen by an applied deformation. The temperature of the specimen was maintained at 7 °C; evaporation was reduced using a vapor trap. The gelation process was monitored until steady state was reached as described (13).

At steady state, we determined the shear amplitude-dependent moduli, \( G(t, \gamma_0) \) by conducting stress-relaxation experiments where the time-dependent stress, \( \sigma(t, \gamma_0) \) (force per unit area), was measured after a step shear deformation of controlled amplitude \( \gamma_0 \) was applied and maintained for 1000 s. The time after application of the step deformation represents a time scale that describes the evolution of the filaments motion in the network (see “Discussion”) and is equivalent to the inverse of the frequency in an oscillatory assay, \( t = 2πf \). The amplitude of the deformation was varied from \( \gamma_0 = 0.5–100 \% \). Since large defor-
nations may break actin filaments, we checked that the network had recovered before applying a new step deformation. A new stress-relaxation experiment was conducted when the overall viscoelastic modulus,

\[ |G^*| = \sqrt{G^2 + G_s^2} \]  

(Eq. 1)

(evaluated at 2\(\pi\) radian/s and \(\gamma_0 = 1\%\), measured during recovery after a stress relaxation measurement, was less than 10% different from the modulus \(G\) (evaluated at 1 s) measured during the 1% amplitude step relaxation experiment. From these stress-relaxation measurements, we extracted \(G(t, \gamma_0) = \sigma(t)/\gamma_0\) as a function of \(\gamma_0\) and time scale \(t\). We also tested the mechanical response of F-actin networks by subjecting them to oscillatory deformations, \(\gamma(t) = \gamma_0 \sin(\omega t)\), of increasing amplitude \(\gamma_0\). The time-dependent stress, \(\sigma(t)\), was measured and plotted as a function of the applied time-dependent deformation \(\gamma(t)\), which produced so-called Lissajous figures (21).

**Finite Element Modeling**—A simple mechanical model, analyzed via the finite element method, was used to supplement the experimental investigations. The model consists of a square plane of orthogonal, equally spaced, cross-linked arrays of individual filaments and filament bundles (Fig. 6, a–c), pretensioned in-plane, and then loaded out of plane. The selected material properties and rigidities of this model network (i.e. \(E_I\), \(E_A\), \(G_J\), see below) are based on published measurements of actin structure and rigidity; the network topology is only approximate and is designed to mimic orthogonal networks such as the actin cortex in non-muscle cells. This model does not incorporate the inherent inhomogeneities of F-actin networks (20). The simulations were conducted using ABAQUS 5.8–16 (HKS 2000). Geometrically non-linear static analysis (large deformations, no inertia terms) was conducted; Newton’s method was used for convergence of the equilibrium equations.

The filament material was modeled as linear elastic with Young’s modulus of \(E = 2.6\) GPa and Poisson’s ratio of \(\nu = 0.4\) (22). The filaments and filament bundles were modeled as Euler-Bernoulli beam elements (23). The cross-sectional properties of these elements are the cross-sectional areas (A), moments of inertia (I), and St. Venant torsional constants (J) (23). These properties for a filament, a 7-filament bundle, and a 19-filament bundle, which were used in the calculations, are given in Table 1. The out-of-plane stiffness of the filament bundle network is resisted by the bending, axial, and torsional stiffness of the filaments and filament bundles. In the linear regime, the bending stiffness of a filament is proportional to \(E/I\), the axial stiffness is proportional to \(E/A\), and the torsional stiffness is proportional to \(G/J\), where \(G = E/[(2(1 + \nu)]\). For an individual filament, \(L\) is the length between supports; for the filament bundle network the effective \(L\) is related to the density of crossings and the rigidity of the crossings, i.e. a bundle is more rigid than a single filament. Perfect connection (no slip) was assumed between any two crossing filaments and/or bundles (24). A small in-plane pretension (1% of the length) was used on the filament and filament bundle network to engage the axial stiffness of the filaments when loaded out-of-plane. The ends of the filaments and bundles were pinned after the application of the pretension. The total out-of-plane loading was constant for all simulations and uniformly distributed on the filaments (i.e. a 7-filament bundle has seven times the load as an individual filament). The response of the center of the model to the loading was monitored.

Individual filaments are 9 nm in diameter, 10 \(\mu\)m long, and are quite slender (\(L/r = 4500\), where \(r\) is the radius if the filament) (Fig. 6a). Filament bundles consist of seven highly packed, perfectly bonded filaments, as depicted in Fig. 6a’. Filament bundles have dramatically higher bending stiffness than a single filament, the mo-

**Fig. 2.** Strain-induced hardening and softening of F-actin/fascin/\(\alpha\)-actinin networks. a and c, relaxation modulus as a function of time scale \(t\) of networks containing 24 \(\mu\)M F-actin and 0.24 \(\mu\)M fascin and 0.24 \(\mu\)M \(\alpha\)-actinin (a) or 0.6 \(\mu\)M fascin and 0.6 \(\mu\)M \(\alpha\)-actinin (c). The amplitude of the step deformation \(\gamma_0\) in each experiment is indicated in the figure. Inset shows the time-dependent stress relaxation modulus for strain amplitudes >10% for the same F-actin/fascin/\(\alpha\)-actinin networks as in a. b and d, modulus of networks containing 24 \(\mu\)M F-actin and 0.24 \(\mu\)M fascin and 0.24 \(\mu\)M \(\alpha\)-actinin (b) or 0.6 \(\mu\)M fascin and 0.6 \(\mu\)M \(\alpha\)-actinin (d) as a function of the deformation amplitude \(\gamma_0\). Symbols in b and d correspond to the time scales 0.05 s (closed squares), 0.1 s (closed circles), 1 s (open squares), 10 s (open circles), and 100 s (closed triangles). Inset in b shows the same modulus as in b but in linear scale. Inset in d shows the same modulus as in d in linear scale.
Synergy of F-actin Cross-linking Proteins

RESULTS

Strain Hardening of F-actin/α-Actinin/Fascin Networks—Using rheological methods, we analyzed the mechanical response of reconstituted F-actin networks in the presence of either α-actinin, fascin, or both. We define a network resilience as the shear amplitude at which the network modulus begins to decrease, presumably due to the breakage and/or alignment of the filaments by the applied stresses (25). To measure the resilience and mechanical response, F-actin filament networks were sequentially subjected to step deformations of increasing amplitude γ₀, which each induced a rapid stress within the network. The subsequent relaxation of the stress, σ(t), was monitored, from which the relaxation modulus, defined as G(t, γ₀) = σ(t)/γ₀, was computed. At small shear amplitudes γ₀, the curves G(t, γ₀) versus t superimposed. This describes the linear regime where the stress σ(t) increases linearly with the input strain amplitude, making the ratio σ(t)/γ₀ independent of γ₀ (Figs. 1 and 2). At large shear amplitudes γ₀, G(t, γ₀) versus t curves did not superimpose anymore (Figs. 1 and 2).

In the absence of cross-linking proteins, actin filament networks exhibited a slight enhancement of their stiffness for increasing strain amplitude as measured by an increase in G for increasing γ₀ (inset, Fig. 1b). F-actin resilience, defined as the ultimate strain γ, at which G evaluated at a fixed time scale t started declining, was ~3% and relatively independent of time scale. Despite its ability to promote extensive filament bundling, fascin was unable to enhance the resilience of F-actin networks (Fig. 1). In the linear regime of small deformation amplitudes, the modulus of F-actin/fascin networks was higher than that of F-actin. The stress relaxation modulus, G(t, γ₀), remained constant for increasingly small deformations until it monotonically decreased with the time scale (Fig. 1, a and c). The modulus G rapidly decreased with strains of amplitude γ₀ > γₑ (Fig. 1, b and d). This phenomenon, shear-induced softening, occurred at all tested time scales between 0.05–100 s, i.e. shear softening was independent of the rate of deformation. Shear softening is due to either the alignment of the filaments with shear or shear-mediated filament breakage or both. The value of the strain amplitude at which the modulus of F-actin started declining, γₑ, decreased for increasing time scales (Fig. 3a). This means that F-actin/fascin networks offered more resilience when sheared rapidly (see “Discussion”). For strain amplitudes larger than γₑ, the rate of shear softening was relatively independent of both the fascin concentration and the rate of strain (Fig. 1, b and d).

In contrast to fascin, α-actinin provided F-actin with additional resistance to shear deformations (13). α-Actinin induced a substantial increase of elasticity particularly at high rates of shear as measured by an increase of G at small deformation amplitudes. Unlike fascin, α-actinin enhanced the modulus for increasing deformation amplitudes (13). This phenomenon, strain hardening, was exacerbated at short time scales, i.e. at time scales shorter than the lifetime of binding of α-actinin to F-actin, because sheared filaments did not have the time to slide past one another (13). This in turn prevented filaments to relax the stress. The yield strain of F-actin networks was augmented up to 8-fold by α-actinin and decreased with time scale (Fig. 3a), i.e. α-actinin enhanced the resilience of F-actin networks, a resilience that increased with the rate of shear.

The trends observed for α-actinin, including strain hardening and enhanced resilience, were amplified by combining α-actinin and fascin. The stress relaxation modulus increased for increasing strain amplitude over a wide range of time scales (Fig. 2, a and c). Accordingly, the shear modulus displayed a dramatic increase for increasing shear amplitudes, a hallmark of strain hardening, before decreasing at large shear ampli-
structures composed of cross-linked networks of filaments and bundles (see "Materials and Methods"). The amount of "filamentous material" employed was constant and equal to 100 × 100 actin filaments. These filaments were symmetrically and orthogonally arranged on a square area of 100 μm², which was supported around the outer edge (see Fig. 6, a and b). The filaments spanned the 100 micrometer distance either as individual filaments or as highly packed perfectly bonded 7-filament bundles (model results were similar for 19-filament bundles). The out-of-plane stiffness of the network of filaments and filament bundles was sensitive to the total number of individual filaments and filament bundles (Fig. 7). The stiffest network consisted neither of all filaments, nor all bundles, but rather a mixture of the two structures (Fig. 7b). Of the networks of filaments and filament bundles, the network of 79 × 79 filaments and 3 × 3 bundles exhibited the greatest initial and tangential stiffness (for 19-filament bundles: 62 × 62 filaments and 2 × 2 bundles had the stiffest response). The mechanical response of the model exhibited two phenomena qualitatively similar to those observed in the experiments: (i) "strain hardening" of the modulus under deformation (Fig. 7a) and (ii) the optimum resilience consisted of a mixed system of cross-linked individual filaments and filament bundles (as opposed to purely filaments or purely bundles) (Fig. 7b). This mechanical behavior grossly corresponds to the cases of F-actin
DISCUSSION

One of the central functions of cross-linked F-actin networks and F-actin bundles is to provide mechanical support to cytoplasm and reinforce cellular protrusions. We investigated potential mechanical synergism between the two cross-linking proteins, α-actinin and fascin, which co-localize to stress fibers and the lamelipodia of adherent cells. In several respects, the mechanical behavior of F-actin/α-actinin/fascin is intermediate between that of F-actin/α-actinin and F-actin/fascin systems. We previously found that the phase angle of F-actin/α-actinin/fascin networks is intermediate between that of the more solid-like F-actin/fascin network and the more liquid-like F-actin/α-actinin network. The dynamics of F-actin cross-linking/bundling mediated by α-actinin and fascin combined is also intermediate between that of fascin and α-actinin.

In several key aspects, however, the combination of α-actinin, actin + α-actinin + fascin, or F-actin + fascin considered above in which combinations that induced some filament bundling and some cross-linking provided an optimum solution, as opposed to individual dopants.

FIG. 5. Response of F-actin/fascin/α-actinin solutions to oscillatory strain of the form γ(t) = γ₀ sin(ωt), a, effect of strain amplitude: Lissajou figures of time-dependent stress σ(t) versus time-dependent strain γ(t) for strain amplitudes of γ₀ = 10, 20, and 30%. b, effect of actin cross-linking protein concentration: Lissajou figures of σ(t) versus γ(t) for increasing concentrations of α-actinin and fascin. The concentrations of α-actinin and fascin were both equal to 0.24 μM (closed squares) and 0.6 μM (closed circles). The arrows indicate an increase in protein concentration. Inset, maximum stress amplitude during shear cycles as a function of cycle number. The total concentration of auxiliary proteins is indicated in the figure. Actin concentration in all experiments is 24 μM.

FIG. 6. Finite element model of F-actin networks. Schematics of the cross-section of an individual actin filament (a), a 7-filament bundle (a'), and a 19-filament bundle (a''). A corner view of representative structures studied in the simulations is shown. These structures contain either filaments (b) or both filaments and filament bundles (b' and b''). These structures contain the same total number of filaments (100 × 100). These filaments form either unbundled or compact bundles, which are symmetrically and orthogonally arranged on a square area of 100 μm² (see also "Materials and Methods"). b, b', and b'' correspond to the case of 100 × 100 filaments, 3 × 3 bundles + 79 × 79 filaments, and 7 × 7 bundles + 51 × 51 filaments, respectively.
Synergy of F-actin Cross-linking Proteins

Table I

| Cross-section area, $A$ | Moment of inertia, $I$ | St. Venant torsion constant, $J$ |
|------------------------|-----------------------|----------------------------------|
| 1 filament              | 64 $\mu m^2$         | 64 $\mu m^4$                     |
| 7-Filament bundle       | 445 $\mu m^2$        | 17,714 $\mu m^4$                |
| 19-Filament bundle      | 1209 $\mu m^2$       | 129,795 $\mu m^4$               |

FIG. 7. Computer-simulated mechanical response of mixed actin structures containing either filaments, filament bundles, or both. (a), strain-dependent response of 100 X 100 individual filaments (middle curve), 79 X 79 individual filaments + 3 X 3 bundles (top curve), and 2 X 2 individual filaments + 14 X 14 bundles (bottom curve). Each bundle contains 7 filaments. (b), low strain amplitude modulus of mixed actin structures for various arrangements of filaments and bundles.

and fascin generate actin filament ultrastructures that synergistically exploit the functions of these proteins. Fascin and $\alpha$-actinin generate F-actin structures that are more resilient and more elastic than produced by fascin and $\alpha$-actinin separately. The enhancement of elasticity of F-actin provided by the combination of fascin and $\alpha$-actinin is spectacularly increased compared with that separately provided by fascin and $\alpha$-actinin and was about 2 orders of magnitude higher than that of suspensions of intermediate filaments (keratin or vimentin) or actin microfilaments (25). Combining fascin and $\alpha$-actinin also greatly enhanced strain-induced hardening and resilience of F-actin.

Structural differences among actin cross-linking proteins initially lead researchers to believe that they differ from one another by their propensity to bundle actin filaments. Compact proteins that embody a tandem of two actin binding domains, such as fascin and fimbrin, would bundle filaments via close packing. Instead, antiparallel homodimers of molecules each containing one actin binding domain separated by a long level arm, such as $\alpha$-actinin and filamin, would induce the formation of orthogonal arrays (2). However, careful biochemical and physical characterization has now made clear that even the compact protein fascin can both cross-link and bundle F-actin at low and high concentrations, respectively (29). Vice versa, the archetypal actin cross-linking proteins $\alpha$-actinin and filamin, which organize F-actin in orthogonal networks at low concentrations, can also bundle actin filaments at high concentrations (30, 31). In support of this dual structural function, immunofluorescence microscopy has shown that fascin localizes not only in actin bundles that support filopodia but also in orthogonal meshworks of the lamellipodia (32, 33). Similarly, $\alpha$-actinin locates not only in the lamellipodia but also in stress fibers (28). Therefore, the roles of cross-linking proteins cannot be only distinguished by their propensity to bundle or cross-link actin filaments in vitro. Instead, we suggest that what also distinguishes cross-linking proteins from one another is their mechanical function. We note that in our experiments we mixed fascin and $\alpha$-actinin first and then added G-actin and the polymerizing salt. We leave to future work the study of how the order of addition of the auxiliary proteins to preassembled F-actin may affect the rheological outcome.

Fascin increases F-actin elasticity at levels well below those observed in living cells (14) and does not help F-actin resist shear stresses of large magnitude. $\alpha$-Actinin greatly enhances the elasticity, resilience, and strain hardening property of F-actin, but not nearly as effectively as $\alpha$-actinin supplemented with fascin. Our results therefore suggest that cells combine $bona fide$ F-actin bundling proteins such as fascin and $bona fide$ cross-linking proteins such as $\alpha$-actinin to produce stiff and resilient cytoskeletal structures.

While the developed finite element model is not an attempt to duplicate or simulate the experiments, it provides an insight into the mechanism by which actin cross-linking proteins may work synergistically. We have recently observed that the combination of the bundling/cross-linking proteins fascin and $\alpha$-actinin induces the formation of mixed structures. Confocal microscopy reveals that these structures contain both highly ordered bundles and orthogonal oriented filaments as opposed to mostly rigid bundles (fascin alone) (29) or mostly orthogonal networks and disorganized bundles ($\alpha$-actinin alone) (20, 26). Our simulations test how these mixed structures may be responsible for the synergistic enhancement of the elasticity of F-actin networks. The existence of an optimum mix of filaments and bundles was by no means obvious from the outset in the mechanical model. Consider that the bending moment of inertia (Table I) for the 7-filament bundles is nearly 8 times greater than the moment of inertia for 7 individual filaments! Thus, an optimum existing of all bundles would seem reasonable. However, as individual filaments are expended to generate the bundles, the density of crossings decreases, weakening the system, in structural terms the “unbraced length” increases as filaments are replaced by bundles. This competition leads to the optimum amount of filaments and bundles shown in Fig. 7b. The simulations suggest that mechanical modeling of networks of filaments and filament bundles can provide additional insight and explanation for the synergistic response of actin cross-linking proteins as measured in living cells.
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