Computational investigation of force generation, relaxation, and remodeling of the actin cytoskeleton

Wonyeong Jung
Purdue University

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By  Wonyeong Jung

Entitled
Computational Investigation of Force Generation, Relaxation, and Remodeling of the Actin Cytoskeleton

For the degree of  Master of Science in Mechanical Engineering

Is approved by the final examining committee:

Taeyoon Kim  
Co-chair
Eric A. Nauman  
Co-chair
Daniel M. Suter

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Approved by Major Professor(s):  Taeyoon Kim and Eric A. Nauman

Approved by:  Jay P. Gore  
Head of the Departmental Graduate Program  
11/22/2016  
Date
COMPUTATIONAL INVESTIGATION OF FORCE GENERATION, RELAXATION, AND REMODELING OF THE ACTIN CYTOSKELETON

A Thesis
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of
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by
Wonyeong Jung

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Requirements for the Degree
of
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5.3 Time evolution of standard deviation of x positions of actins \((\sigma_{x})\) for the cases shown in Figure 5.2. ACP density used in these cases is (a) 0.01, (b) 0.018, (c) 0.032, (d) 0.056, and (e) 0.1. Motor density is 0.0008 (red), 0.0026 (blue), 0.008 (green), 0.026 (cyan), and 0.08 (black). (All parts were reprinted from [23].)

5.4 Buckling of actin filaments in the case shown in Figure 5.2b. (a) The ratio of end-to-end distance to contour length of two selected actin filaments. The actin filament represented by red experienced a sequence of buckling events at around \(t = 8, 25, 50,\) and \(80\) s, whereas the actin filament represented by blue underwent buckling at around \(t = 8\) s and was straightened at around \(t = 75\) s. (b, c) Visualization of (b) subsequent buckling events and (c) straightening of buckled actin filaments shown in (a). Solid circles located at the ends of the actin filaments represent their barbed ends. (All parts were reprinted from [23].)
5.5 Buckling of actin filaments plays a crucial role in bundle formation and tension generation. (a) Number of actin filaments that experience buckling at least once during simulation depending on densities of motors \( R_M \) and ACPs \( R_{ACP} \), normalized by the largest number. (b-c) Snapshots showing actin density of networks where buckling is suppressed via a 100-fold increase in bending stiffness of actin filaments \( \kappa_{b,A} \). (d) Time evolution of generated tension (solid line) and the number of buckling events (dashed line) for cases with reference bending stiffness (blue triangle, \( \kappa_{b,A} = \kappa_{b,A}^* \)) and 100-fold higher bending stiffness (red circle, \( \kappa_{b,A} = 100 \times \kappa_{b,A}^* \)) at \( R_M = 0.08 \) and \( R_{ACP} = 0.1 \). (e) Distribution of forces exerted on motors \( f_{\text{max}}^M \) and ACPs \( f_{\text{max}}^{ACP} \) measured at peak tension for cases shown in (d). Legend is shared with (d). (f) The maximum and (g) sustainability of tension measured from cases \( \kappa_{b,A} = 100 \times \kappa_{b,A}^* \) with various \( R_M \) and \( R_{ACP} \). (h) Compaction time. (i) Standard deviation of x positions of actins at compaction time \( \sigma_x^c \). (All parts were reprinted from [23].)

5.6 Effects of duty ratio of motors on bundle formation and tension generation. Densities of motors and ACPs are 0.08 and 0.1, respectively. Compared to the reference case with same \( R_M \) and \( R_{ACP} \) (Figure 5.2b), stall force of motors was decreased from 5.7 pN to 5.3 pN, and unbinding rate was increased from 0.049 s\(^{-1}\) to 0.49 s\(^{-1}\). (a) Snapshots showing actin density in the networks at \( t = 10, 30, \) and 60 s. A bundle forms well as in the reference case. Time evolution of (b) tension and (c) standard deviation of x positions of actins \( \sigma_x \) which shows similar tendency to that in the reference case. (All parts were reprinted from [23].)

5.7 Time evolution of standard deviation of x positions of actins \( \sigma_x \) for the cases shown in Figure 5.5. Density of ACPs used in these cases is (a) 0.01, (b) 0.032, and (c) 0.1. Motor density is 0.0008 (red), 0.0026 (blue), 0.008 (green), 0.026 (cyan), and 0.08 (black). (All parts were reprinted from [23].)
5.8 Densities of motors ($R_M$) and ACPs ($R_{ACP}$) used in cases shown here are 0.08 and 0.01, respectively. (a-c) (1st column) Orientations where barbed ends of actin filaments in networks are initially directed. Red on the circles located at the bottom-right corner represents the range of the orientation. Arrows in the boxes represent examples of filaments with corresponding initial orientations. (2nd, 3rd, 4th columns) Snapshots showing actin density in the networks at $t = 10, 30,$ and $60$ s with initial orientation indicated in the 1st column. (5th column) Initial and final orientations of actin filaments. Final orientation indicates orientation of filaments measured at a time point when compaction time is defined. (d) Time evolution of tension for cases with biased initial orientations shown in (a-c) and isotropic initial orientation. (e) Number of buckling events occurring during simulation for cases shown in (d). (f) Time evolution of a fraction of antiparallel filament pairs for cases shown in (d). (All parts were reprinted from [23].)  

5.9 Rotation of actin filaments in the case shown in Figure 5.8c. (a) Time evolution of orientation of two selected actin filaments. At around $t = 20$ s, both actin filaments rotate by about $180^\circ$ (b, c) Visualization of rotation of the actin filaments shown in (a). Solid circles located at the ends of the actin filaments represent their barbed ends. (All parts were reprinted from [23].)  

5.10 Effects of initial orientation of diagonally nuclearized actin filaments on bundle formation and tension generation. Densities of motors and ACPs used in cases shown here are 0.08 and 0.01, respectively. (a-d) (1st column) Orientations where barbed ends of actin filaments in networks are initially directed. Red lines on the circles located at the bottom-right corner represent the orientations. Arrows in the boxes represent examples of filaments with corresponding initial orientations. (2nd, 3rd, 4th columns) Snapshots showing actin density in the networks at $t = 0, 10,$ and $40$ s with initial orientation indicated in the 1st column. (e) Time evolution of tension for cases shown in (a-d). (f) Time evolution of orientations of selected actin filaments in the case shown in (a). (g) Time evolution of a fraction of antiparallel filament pairs for cases shown in (a-d). (h) Number of buckling events occurring during simulations for cases shown in (a-d). (All parts were reprinted from [23].)
5.11 Influences of initial orientation of perpendicularly nuclearized actin filaments on bundle formation and tension generation. Densities of motors and ACPs used in cases shown here are 0.08 and 0.01, respectively. (a-d) (1st column) Orientations where barbed ends of actin filaments in networks are initially directed. (2nd, 3rd, 4th columns) Snapshots showing actin density in the networks at \( t = 0, 10, \) and \( 40 \) s with initial orientation indicated in the 1st column. (e) Time evolution of tension for cases shown in (a-d). (f) Time evolution of orientation of selected actin filaments from case shown in (a). (g) Time evolution of a fraction of antiparallel filament pairs for cases shown in (a-d). (h) Number of buckling events occurring during simulations for cases shown in (a-d). (All parts were reprinted from [23].) 

5.12 Effects of initial orientation of actin filaments on bundle formation and tension generation in networks with numerous ACPs. Densities of motors and ACPs used in cases shown here are 0.08 and 0.1, respectively. (a-c) (1st column) Orientations where barbed ends of actin filaments in networks are initially directed. (2nd, 3rd, 4th columns) Snapshots showing actin density in the networks at \( t = 10, 30, \) and \( 60 \) s with initial orientation indicated in the 1st column. (5th column) Initial and final orientations of actin filaments. Final orientation indicates orientation of filaments measured at a time point when compaction time is defined. (d) Time evolution of tension for cases with biased initial orientations shown in (a-c) and isotropic initial orientation. (e) Time evolution of a fraction of antiparallel filament pairs for cases shown in (d). (All parts were reprinted from [23].) 

5.13 In networks with biased filament orientation, parallel filament pairs can form bundles without buckling of filaments, whereas antiparallel pairs cannot. Densities of motors \( (R_M) \) and ACPs \( (R_{ACP}) \) are 0.08 and 0.01, respectively as in Figure 5.8, but bending stiffness of actin filaments is increased 100-fold \( (100 \times \kappa_{b,A}^*). \) (a-c) (1st column) Initial orientations of actin filaments in networks. (2nd, 3rd, 4th columns) Snapshots showing actin density in the networks with initial filament orientation indicated in the 1st column. (5th column) Initial and final orientation of actin filaments. (d) Time evolution of tensile forces generated by bundles for cases with biased initial orientations shown in (a-c) and isotropic initial orientation. (e) Time evolution of a fraction of antiparallel filament pairs for cases shown in (d). (All parts were reprinted from [23].)
In networks with biased filament orientations, bundles can form without buckling of actin filaments. Schematic diagrams show actin filaments and motors initially directed toward (a) \(+x/+y\), (b) \(+x/\pm y\), and (c) \(x/+y\) as in cases shown in Figures 5.8a-c. Teal and red represent actin filaments and motors, respectively, whereas ACPs are not shown for simplicity. (a) Most of the actin filaments oriented toward \(+x/+y\) are aligned in parallel via polarity sorting. (b) Antiparallel pairs of actin filaments initially oriented relatively in the y-direction can be aligned well in the y-direction. However, the alignment results in the buildup of compressive forces on the actin filaments unlike in other cases. If bending stiffness of actin filaments is low enough, the actin filaments are buckled and oriented in the y-direction. If buckling is suppressed due to large bending stiffness, the actin filaments cannot be oriented in the y-direction. (c) Antiparallel pairs of actin filaments initially oriented relatively in the x-direction can be aligned in the y-direction via polarity sorting. (All parts were reprinted from [23].)

Densities of motors (\(R_M\)) and ACPs (\(R_{ACP}\)) used in cases shown here are 0.08 and 0.01, respectively. (a-d) Snapshots showing actin density in networks (a) without actin turnover and (b-d) with actin turnover rate (\(k_{t,A}\)) of 60 s\(^{-1}\). In networks with actin turnover, depolymerization of actin filaments was inhibited by bound ACPs or motors to an extent determined by inhibition factor (\(\zeta_{d,A}\)). \(\zeta_{d,A}\) ranges between 0 (no inhibition of depolymerization) and 1 (complete inhibition). In these examples, \(\zeta_{d,A}\) is (b) 0, (c) 0.6, or (d) 1. (e) Time evolution of tensile forces generated by bundles for cases shown in (a-d). (f) The maximum and (g) sustainability of tension, depending on \(k_{t,A}\) and \(\zeta_{d,A}\). Maximum tension shows no correlation with \(k_{t,A}\) and \(\zeta_{d,A}\), whereas sustainability is higher at intermediate range of \(\zeta_{d,A}\). (h) Compaction time. (i) Standard deviation of x positions of actins (\(\sigma_x\)) at compaction time. With more turnover (i.e. high \(k_{t,A}\) and low \(\zeta_{d,A}\)), bundles form faster, but the formed bundles are more loose. (All parts were reprinted from [23].)

Time evolution of standard deviation of x positions of actins (\(\sigma_x\)) in the cases shown in Figure 5.15. The turnover rate used in these cases is (a) 30 s\(^{-1}\), (b) 45 s\(^{-1}\), (c) 60 s\(^{-1}\), (d) 75 s\(^{-1}\), and (e) 90 s\(^{-1}\). The inhibition factor is 0 (red), 0.2 (blue), 0.4 (green), 0.6 (cyan), 0.8 (black), and 1 (magenta). (All parts were reprinted from [23].)
5.17 Impacts of actin turnover on bundle formation and tension generation in networks with numerous ACPs. Densities of motors and ACPs used in cases shown here are 0.08 and 0.1, respectively. (a-d) Snapshots showing actin density in networks (a) without actin turnover and (b-d) with actin turnover rate ($k_{t,A}$) of 60 s$^{-1}$. In networks with actin turnover, depolymerization of actin filaments was inhibited by bound ACPs or motors to an extent determined by inhibition factor ($\zeta_{d,A}$). $\zeta_{d,A}$ ranges between 0 (no inhibition of depolymerization) and 1 (complete inhibition). In these examples, $\zeta_{d,A}$ is (b) 0, (c) 0.5, or (d) 1. (e) Time evolution of tensile forces generated by bundles for cases shown in (a-d). (f) Distribution of forces exerted on motors ($f_{M}^{\text{max}}$) and ACPs ($f_{\text{ACP}}^{\text{max}}$) measured at peak tension for cases shown in (a-d). The gray dashed line indicates stall force of motors (5.7pN). The legend is shared with (e). (g) The maximum and (h) sustainability of tension, depending on $k_{t,A}$ and $\zeta_{d,A}$. (i) Compaction time. (j) Standard deviation of x positions of actins at the compaction time ($\sigma_{x}^{c}$). (All parts were reprinted from [23].)

5.18 Time evolution of standard deviation of x positions of actins ($\sigma_{x}$) in the cases shown in Figure 5.17. The turnover rate used in these cases is (a) 60 s$^{-1}$, (b) 90 s$^{-1}$, and (c) 120 s$^{-1}$. The inhibition factor is 0 (red), 0.25 (blue), 0.5 (green), 0.75 (cyan), and 1 (black). (All parts were reprinted from [23].)
Diverse mechanics of the F-actin cytoskeleton mediate essential behaviors of cells, including cell division, migration, and shape change. Force generation by motor proteins and the resultant morphological change of cytoskeletal networks govern cellular processes such as migration and division. Cell stiffening and softening under external mechanical stimuli regulate cell shape. In this thesis, interplay between various cytoskeletal components during these processes is investigated using an agent-based computational model to elucidate mechanical factors underlying these processes. This thesis is composed of three independent studies.

First, force generation in cortical actomyosin networks is studied. Using the computational model, the effects of motor activity and the density and kinetics of actin cross-linking proteins (ACPs) on the accumulation and maintenance of mechanical tension are quantitatively determined. We show that motors accumulate large stress quickly by behaving as temporary cross-linkers although this stress is relaxed over time unless there are sufficient passive ACPs to stabilize the network. Stabilization by ACPs helps motors to generate forces up to their maximum potential, significantly enhancing efficiency and stability of stress generation. Thus, it is demonstrated that the force-dependent kinetics of ACP dissociation plays a critical role in the accumulation and sustainment of stress and the structural remodeling of networks.

Second, molecular origin of stress relaxation in cross-linked actin networks under shear strain is investigated. To date, stress relaxation has been mainly attributed to the transient nature of ACPs that connect F-actins. By contrast, potential effects of
rich F-actin dynamics on stress relaxation have been neglected in most previous studies. In this study, it is demonstrated that F-actin severing arising from compression-induced filament buckling coordinates with ACP unbinding, leading to very distinct modes of stress relaxation. Furthermore, conditions under which the F-actin severing dominates the mechanical response are established, providing additional mechanistic insight into the viscoelasticity of the F-actin cytoskeleton.

Third, formation of transverse arcs from actomyosin networks is studied. Transverse arcs form via actomyosin-driven condensation of F-actins in the lamellipodia of migrating cells and exerts significant forces on the surrounding environments. Structural reorganization of a network into a bundle facilitated by actomyosin contractility is a physiologically relevant and biophysically interesting process. Nevertheless, it remains unclear how F-actins are reoriented, buckled, and bundled as well as undergo tension buildup during the structural reorganization. In this study, how the interplay between the density of myosin motors and ACPs and the rigidity, initial orientation, and turnover of F-actins regulate the reorganization process is demonstrated.
1. INTRODUCTION

Cells are a basic component of all living organisms. Their ability to reproduce, move, and sense the extracellular environment maintains and organizes the everyday life of living things. Cells have very different functions and organizations depending on the cell type. These differences are provided by the cytoskeleton (Figure 1.1), which is a structural framework of cells composed of interlinking filamentous proteins [1]. Since the cytoskeleton extends throughout a cell connecting the cell membrane and internal organelles, its organization determines and maintains cell shape. In addition, the cytoskeleton is very dynamic, being able to reorganize in less than a minute [1], which gives rise to various mechanical behaviors of cells. For example, molecular motors inside the cytoskeleton generate force, which is needed for muscle contraction, cell migration, and cell division. In addition, cells can sense and respond to external mechanical stimuli. For example, cells under external stress, such as shear stress generated by the blood flow, can maintain their shape by stiffening their cytoskeletons [2]. Thus, investigating mechanical behavior of the cytoskeleton is a key to understand these cellular processes.

The cytoskeleton comprises three main filamentous systems: microfilaments (the actin cytoskeleton), microtubules, and intermediate filaments [1]. This thesis focuses on mechanical behaviors of the actin cytoskeleton. In section 1.1 and 1.2, the components of the actin cytoskeleton—actin and accessory proteins including actin cross-linking proteins and myosins—will be introduced. In section 1.3, an overview of this thesis will be provided.
1.1 Actins

1.1.1 Structure and assembly of actins

The main component of the actin cytoskeleton is actin, which is a protein found in eukaryotic cells. The actin monomer is a globular protein, called G-actin. G-actin has an asymmetric structure, with a cleft in its center where ATP or ADP along with Mg$^{2+}$ can bind. G-actins can self-assemble into a double-stranded polymer, F-actin (Figure 1.2). F-actin exhibits polarity, with the ATP binding cleft of G-actin subunits pointing toward one end of an F-actin, which is called the “(-) end” or the “pointed end.” The other end of an F-actin is called “(+) end” or the “barbed end.” F-actin has a diameter of 7 ∼ 9 nm and length of about 0.1 ∼ 20 µm [3].

When F-actin assembles in vitro, two or three G-actins first assemble spontaneously if there are sufficient amount of G-actins. After that, more G-actins bind to the assembly, elongating it rapidly. It has been found that association and dissociation rates of G-actins are different in the barbed and the pointed end; association rate at the barbed end is about ten times higher than that at the pointed end, while dissociation rate at the barbed end is about two times higher than that at the pointed end.
end [1]. Thus, at steady-state, F-actin gains G-actins at the barbed end and loses G-actins at the pointed end. This phenomenon is called “treadmilling.” Studies have demonstrated that treadmilling plays a crucial role in cell motility [4,5].

1.1.2 Mechanical properties of actins

The F-actin is a semiflexible polymer, meaning that the persistence length, the length over which thermal bending of a polymer becomes appreciable [3], is comparable to the typical length of F-actins. Thus, F-actin can be bent by thermal forces, which is called the thermal fluctuation. The semiflexibility governs non-linear force-extension relationships of the actin cytoskeleton, which plays a significant role in various situations. For example, the actin cytoskeleton of cells comprising tissues stiffen under large strain; this prevents large deformation of cells, which harms tissue integrity [6].
1.2 Accessory proteins

In cells, dynamics and structure of the actin cytoskeleton are governed by various kinds of accessory proteins. For example, actin nucleating proteins, including formin and Arp2/3 complex, initiate the assembly of F-actins. Formin assembles long F-actins, whereas Arp2/3 complex assembles branched actin networks. In addition, actin treadmilling is regulated by several kinds of proteins, such as profilin, cofilin, and capping proteins. Profilin facilitates actin treadmilling by converting ADP-bound actin to ATP-bound actin, which is more favorable for F-actin assembly. Cofilin binds to F-actins and changes mechanical properties of actins, making them prone to being severed [7]. Severing of F-actins induced by cofilin also facilitates actin treadmilling owing to the generation of free ends of F-actins. On the other hand, capping proteins, such as CapZ, tropomodulin, and gelsolin, hinder actin treadmilling by binding to either barbed or pointed ends of F-actins. Another kind of accessory proteins is actin cross-linking proteins (ACPs). ACPs connect two F-actins to organize various kinds of actin structures in cells (Figure 1.3). The actin structures can perform distinct functions owing to the activity of myosins, a family of motor proteins, within these structures. Myosins can move along F-actins to transport cellular cargos or to generate force needed for cellular processes such as cell migration or cell division. In the following subsections, ACPs and myosins will be introduced in more detail.

1.2.1 Actin cross-linking proteins

ACPs have two binding sites which can bind to F-actins through electrostatic interaction [8], thus connecting F-actins pairs. Different kinds of ACPs form various actin structures that can be found in different locations in cells (Figure 1.3). Tightly packed actin bundles can be found in filopodia, in stress fibers, or in contractile rings [9]. On the other hand, actin networks can be found in leading edges of migrating cells or in cell cortices. It has been found that formin and small ACPs such as fimbrin, scruin, fascin, or espin tend to form bundles [1,8], whereas Arp 2/3 complex and larger
ACPs such as filamin have been shown to form networks [1,8]. Examples of a bundle formed by fimbrin and a network formed by filamin are shown in Figure 1.4.

Figure 1.4. (a) An actin actin bundle (bottom) formed by fimbrin (top). (b) An actin network (bottom) formed by filamin (top). Binding sites of fimbrin and filamin are marked with blue. (All parts were reprinted from [1].)
1.2.2 Unbinding of actin cross-linking proteins

Cross-links formed by ACPs are transient; ACPs bound to F-actins can unbind with unbinding rate on the order of $1 \text{s}^{-1}$ at room temperature [8]. Moreover, unbinding of ACPs can be facilitated by force exerting on them. It has been found that unbinding rate of ACPs increases exponentially as force exertion increases, which is called a “slip-bond” behavior [10]. The transient nature of ACP allows reorganization of actin cytoskeleton, which is indispensable in cellular processes that involve dramatic change in cell shape, such as migration or division.

1.2.3 Myosins

Myosins can move along F-actins by harnessing energy released from ATP hydrolysis. There are several types of myosins that perform various functions in cells. For example, myosin II generates contractile force for muscle contraction or cellular processes including migration and division. Another protein, myosin V, transports cellular cargo along F-actins. In addition, myosin I is known to link the actin cytoskeleton to membrane or to be involved in endocytosis [1]. In this thesis, we focused on roles of myosin II in the actin cytoskeleton. Structure and dynamics of myosin II will be explained in the following subsections.

1.2.4 Structure of myosin II

Myosin II consists of two heavy chains and four light chains (Figure 1.5). A heavy chain is composed of head, neck, and tail parts. Head parts contain actin binding sites and nucleotide binding sites responsible for ATP hydrolysis [1]. Myosin II molecules assemble into a larger structure called thick filament, which is shown in the center of Figure 1.6. About 56 to 800 of myosin II molecules assemble into thick filaments whose length ranging from about 0.3 $\mu\text{m}$ to 1.5 $\mu\text{m}$ [11]. The length of a thick filament and number of myosin II molecules inside the filament differ depending on the cell.
type. Thick filaments in skeletal muscle cells are longer and have more myosin II molecules than those in non-muscle cells.

1.2.5 Dynamics of myosin II

Myosin II walks toward barbed ends of F-actins, harnessing energy from ATP hydrolysis through a process called the “cross-bridge cycle,” which consists of the following steps [1,12]: (1) ATP binds to myosin that was attached to actin, releasing myosin from the actin. (2) ATP hydrolysis occurs, and myosin stores the energy from the hydrolysis by changing its conformation. (3) Myosin head binds to actin again, and P_i releases from the myosin head. This makes the myosin head return to its original conformation. By changing back to the original conformation, myosin pushes
actin toward its pointed end, thus moving toward the barbed end. (4) ADP releases and ATP binds to myosin. The cycle repeats.

When myosin connects antiparallel filaments as shown in Figure 1.6, myosin can generate contractile force on the structure. Skeletal muscle cells contain repeating arrays of such structure to effectively generate contractile force. In non-muscle cells, myosin-induced contraction is indispensable in various biological processes. For example, it regulates cell shape and provides traction force during cell migration.

1.3 Thesis Overview

This thesis consists of three studies. In the first study, effects of myosin motor and ACP activity on force generation and morphology of actomyosin networks mimicking the cell cortex were investigated. It was found that with a sufficient amount of ACPs, motors can generate stable force up to their full potential, but the force relaxes over time and networks remodel significantly if there are insufficient amounts of ACPs. In the second study, impacts of F-actin fragmentation on mechanical responses of cross-linked actin networks were investigated. It was found that when actin networks are subjected to shear strain, F-actins are buckled and thus severed, leading to the stress relaxation of the networks. In addition, the effects of severing were compared to the effects of ACP unbinding that had been believed to be a main source of stress relaxation. We found that F-actin severing and ACP unbinding occur in different locations, leading to distinct modes of stress relaxation. In the third study, regulating factors of transformation of thin actomyosin networks to bundles, mimicking transformation of lamellipodial actin networks into transverse arcs, were investigated. It was found that densities of motors and ACPs, buckling events of F-actins, initial orientation of F-actins, and F-actin turnover govern the formation of bundles and tension buildup during the bundle formation process.
2. METHODS

(Most of this section is adopted from [13,14])

To study force generation, relaxation, and remodeling of the actin cytoskeleton, we performed Brownian dynamics simulations of cytoskeletal networks using an agent-based computational model [15–17]. In the computational model [15–17], cytoskeletal components—F-actins, ACPs, and motors—are simplified into cylindrical spring segments connected by elastic hinges. ACPs and motors mimic geometries and mechanical properties of α-actinin [18] and non-muscle myosin II [19], respectively. Figure 2.1 shows the cytoskeletal components of the computational model. Parameters and their reference values used in the model are listed in Table 2.1.

Table 2.1. List of parameters employed in the model.

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<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r_{0,A}$</td>
<td>Length of an actin segment</td>
<td>$1.4 \times 10^{-7}$ (m)</td>
</tr>
<tr>
<td>$r_{c,A}$</td>
<td>Diameter of an actin segment</td>
<td>$4.0 \times 10^{-9}$ (m) [20]</td>
</tr>
<tr>
<td>$\theta_{0,A}$</td>
<td>Bending angle of F-actin</td>
<td>0 (rad)</td>
</tr>
<tr>
<td>$\kappa_{s,A}$</td>
<td>Extensional stiffness of F-actin</td>
<td>$1.69 \times 10^{-2}$ (N/m)</td>
</tr>
<tr>
<td>$\kappa_{b,A}$</td>
<td>Bending stiffness of F-actin</td>
<td>$2.64 \times 10^{-19}$ (N-m) [21]</td>
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<td>Length of an ACP arm</td>
<td>$2.35 \times 10^{-8}$ (m) [22]</td>
</tr>
<tr>
<td>$r_{c,ACP}$</td>
<td>Diameter of an ACP arm</td>
<td>$1.0 \times 10^{-8}$ (m)</td>
</tr>
<tr>
<td>$\theta_{0,ACP}$</td>
<td>Bending angle formed by two ACP arms</td>
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<tr>
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<td>Length of a bare zone of motor backbone</td>
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<td>$r_{b,M2}$</td>
<td>Length of a side segment of motor backbone</td>
<td>$4.2 \times 10^{-8}$ (m)</td>
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*continued on next page*
Table 2.1. continued

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Value</th>
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<tbody>
<tr>
<td>$\theta_{0,M}$</td>
<td>Bending angle of motor backbone</td>
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<td>$\kappa_{s,M1}$</td>
<td>Extensional stiffness of a bare zone</td>
<td>$1.69 \times 10^{-2}$ (N/m)</td>
</tr>
<tr>
<td>$\kappa_{s,M2}$</td>
<td>Extensional stiffness of a side segment</td>
<td>$1.69 \times 10^{-2}$ (N/m)</td>
</tr>
<tr>
<td>$\kappa_{b,M}$</td>
<td>Bending stiffness of motor backbone</td>
<td>$5.07 \times 10^{-18}$ (N·m)</td>
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<td>Length of a motor arm</td>
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<tr>
<td>$r_{c,M}$</td>
<td>Diameter of a motor arm</td>
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</tr>
<tr>
<td>$\kappa_{s,M4}$</td>
<td>Extensional stiffness 2 of a motor arm</td>
<td>$1.0 \times 10^{-3}$ (N/m)</td>
</tr>
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<td>$N_h$</td>
<td>Number of heads represented by a motor arm</td>
<td>8</td>
</tr>
<tr>
<td>$N_a$</td>
<td>Number of arms per motor</td>
<td>8</td>
</tr>
<tr>
<td>$\kappa_r$</td>
<td>Strength of repulsive force</td>
<td>$1.69 \times 10^{-3}$ (N/m)</td>
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<tr>
<td>$C_A$</td>
<td>Actin concentration</td>
<td>20-25 ($\mu$m)</td>
</tr>
<tr>
<td>$R_M$</td>
<td>Ratio of motor concentration to $C_A$</td>
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</tr>
<tr>
<td>$R_{ACP}$</td>
<td>Ratio of ACP concentration to $C_A$</td>
<td>0.001-0.2</td>
</tr>
<tr>
<td>$&lt;L_f&gt;$</td>
<td>Average length of F-actins</td>
<td>1.1-1.44 ($\mu$m)</td>
</tr>
<tr>
<td>$\Delta t$</td>
<td>Time step</td>
<td>$2.3 \times 10^{-6}$ (s)</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Viscosity of medium</td>
<td>$8.6 \times 10^{-2}$ (kg/m·s)</td>
</tr>
<tr>
<td>$k_{u,ACP}^0$</td>
<td>Zero-force unbinding rate coefficient of ACP</td>
<td>$0.115$ (s$^{-1}$) (= $k_{u,ACP}^0$) [18]</td>
</tr>
<tr>
<td>$\lambda_{u,ACP}$</td>
<td>Force sensitivity of ACP unbinding</td>
<td>$1.04 \times 10^{-10}$ (m) [18]</td>
</tr>
<tr>
<td>$k_B T$</td>
<td>Thermal energy</td>
<td>$4.142 \times 10^{-21}$ (J)</td>
</tr>
<tr>
<td>$E$</td>
<td>Youngs modulus of an elastic substrate</td>
<td>$3.0 \times 10^4$ (Pa)</td>
</tr>
</tbody>
</table>

2.1 Mechanics of F-actins, ACPs, and Motors

F-actins are modeled as a series of cylindrical segments with fixed polarity (barbed and pointed ends) whose length and diameter are 140 nm and 7 nm respectively,
An agent-based computational model was employed to study force generation, relaxation, and remodeling of the actin cytoskeleton. Actin filaments, motors, and ACPs are simplified into cylindrical segments connected by elastic hinges. Actin filaments (blue) are modeled as a series of cylindrical segments with polarity (barbed and pointed ends). Motors (red) consist of a backbone with symmetric polarity and arms representing myosin motor heads. ACPs (yellow) are modeled as two parallel arms. Arms of motors and ACPs can bind to actin filaments. Equilibrium lengths of segments of actin filaments (A), motors (M), and ACPs are maintained by extensional stiffness ($\kappa_s$), whereas equilibrium angles between segments are maintained by bending stiffness ($\kappa_b$). (Reprinted from [23].)

connected by elastic hinges (Figure 2.1). ACPs are modeled as pairs of cylindrical arms of 23.5 nm in length and 10 nm in diameter, connected to each other by elastic hinges. Motors mimic the structure of myosin bipolar thick filaments (TF) [19] with a relatively rigid backbone with symmetric polarity comprising a bare zone of 42 nm in length at center and multiple segments of 42 nm in length connected by hinges. Each endpoint of the backbone segment has two motor arms. Each of the motor arms attached to the backbone represents $N_h$ myosin heads. In a previous study [24], it was demonstrated that arms of a motor are mechanically coupled via the backbone so that the motor behaves as myosin TF with $N_a N_h$ heads, where $N_a$ is the number of arms. Both $N_h$ and $N_a$ are set to 8, corresponding to 64 myosin heads per TF.
which is comparable to the experimentally determined size of non-muscle myosin thick filaments [25].

The arms of ACPs and motors can bind to binding sites located every 7 nm on actin segments. Motions of segments representing F-actins, ACPs, and motors are governed by the Langevin equation with inertia neglected:

\[ \mathbf{F}_i - \zeta_i \frac{d\mathbf{r}_i}{dt} + \mathbf{F}_i^T = 0 \]  \hspace{1cm} (2.1)

where \( \zeta_i \) is a drag coefficient, \( \mathbf{r}_i \) is the position of either center point of ACPs or endpoint of segments constituting F-actin and motor backbones, \( t \) is time, and \( \mathbf{F}_i \) is a net deterministic force which includes extensional, bending, and repulsive forces. \( \mathbf{F}_i^T \) is a thermal force determined by the fluctuation-dissipation theorem:

\[ \langle \mathbf{F}_i^T(t)\mathbf{F}_j^T(t) \rangle = \frac{2k_B T \zeta_i \delta_{ij}}{\Delta t} \boldsymbol{\delta} \]  \hspace{1cm} (2.2)

where \( \delta_{ij} \) is the Kronecker delta, \( \Delta t \) is time step, and \( \boldsymbol{\delta} \) is a unit second-order tensor. Approximated drag coefficients of actin segment or ACP [26] are represented by:

\[ \zeta_i = 3\pi \mu r_{c,i}^3 + \frac{r_{0,i}}{r_{c,i}^5} \]  \hspace{1cm} (2.3)

where \( \mu \) is the viscosity of medium, and \( r_{c,i} \) and \( r_{0,i} \) are length and diameter of actin segment or ACP arm, respectively. Using the Euler integration scheme, the position of each actin segment or ACP is updated at each time step:

\[ \mathbf{r}_i(t + \Delta t) = \mathbf{r}_i(t) + \frac{d\mathbf{r}_i}{dt} \Delta t = \mathbf{r}_i(t) + \frac{1}{\zeta_i} (\mathbf{F}_i + \mathbf{F}_i^T) \Delta t \]  \hspace{1cm} (2.4)

The sum of deterministic forces and stochastic force determines velocity of each segment that is then used in the Euler integration scheme to update positions of segments. The deterministic forces include extensional, bending, and repulsive forces described by harmonic potentials.
Extension and bending of actins, ACPs, and motors are governed by the following harmonic potentials:

\[ U_s = \frac{1}{2} \kappa_s (r - r_0)^2 \]  \hspace{1cm} (2.5)

and

\[ U_b = \frac{1}{2} \kappa_b (\theta - \theta_0)^2 \]  \hspace{1cm} (2.6)

where \( r \) is a length of actin segment, ACP arm or motor arm, \( \kappa_s \) is an extensional stiffness, \( \theta \) is a bending angle, \( \kappa_b \) is a bending stiffness, and the subscript 0 indicates an equilibrium value. The value of actin bending stiffness \( (\kappa_{b,A}) \) corresponds to the persistence length of 9 \( \mu \)m [21].

A repulsive force between actin segments is represented by a harmonic potential [15]:

\[
U_r = \begin{cases} 
\frac{1}{2} \kappa_r (r_{12} - r_{c,A})^2 & \text{if } r_{12} < r_{c,A} \\
0 & \text{if } r_{12} \geq r_{c,A}
\end{cases}
\]  \hspace{1cm} (2.7)

where \( \kappa_r \) is the strength of repulsive force, and \( r_{12} \) is a minimum distance between two actin segments.

### 2.2 Dynamics of ACPs

ACP bind to binding sites on F-actins without preference for angle of contact, leading to formation of homogeneous networks at low density and bundles at high density, as observed in networks cross-linked by filamin A [27]. The binding site is located every 7nm on an actin segment. ACPs unbind with a rate:

\[
k_u = \begin{cases} 
    k_u^0 \exp\left(\frac{\lambda_u |F_{s,ACP}|}{k_B T}\right) & \text{if } r \geq r_{0,ACP} \\
    k_u^0 & \text{if } r < r_{0,ACP}
\end{cases}
\]  \hspace{1cm} (2.8)

where \( F_{s,ACP} \) is a tensile force exerted on an ACP arm, \( k_u^0 \) is a zero-force unbinding rate coefficient, \( \lambda_u \) is compliance of a bond for ACP unbinding, and \( k_B T \) is thermal
energy. The reference values of $k_0^u = 0.115 \text{ s}^{-1}$ and $\lambda_u = 1.04 \times 10^{-10} \text{ m}$ are adopted from a single-molecule experiment using filamin A [18].

### 2.3 Dynamics of Motors

Walking $(k_{w,M})$ and unbinding rates $(k_{u,M})$ of motor arms are determined by the “parallel cluster model” (PCM) [28,29]. Table 2.2 shows values for the major parameters used in the model to adopt PCM. It was assumed that only forces acting on the longitudinal spring of the motor arms ($\vec{F}_{s,M4} = \nabla U_{s,M4}$) affect $k_{w,M}$ and $k_{u,M}$. At each walking event, arms slide from a current binding site to a next one located toward the barbed end by 7 nm. After reaching the barbed end, motors slide off from F-actin via a next walking event. Note that it is assumed that myosin heads behave as a catch bond [30,31], leading to lower $k_{w,M}$ and $k_{u,M}$ with higher applied forces (Figure 2.2). We used mechanochemical rates for motors which result in unloaded walking velocity of 140 nm/s and stall force of $f_{M}^{\text{stall}} \sim 5.7 \text{ pN}$.

Table 2.2. List of parameter values employed to adopt the parallel cluster model [28,29].

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{01}$</td>
<td>A rate from unbound to weakly bound state</td>
<td>40 (s$^{-1}$)</td>
</tr>
<tr>
<td>$k_{10}$</td>
<td>A rate from weakly bound to unbound state</td>
<td>2 (s$^{-1}$)</td>
</tr>
<tr>
<td>$k_{12}$</td>
<td>A rate from weakly bound to post-power-stroke state</td>
<td>1000 (s$^{-1}$)</td>
</tr>
<tr>
<td>$k_{21}$</td>
<td>A rate from post-power-stroke to weakly bound state</td>
<td>1000 (s$^{-1}$)</td>
</tr>
<tr>
<td>$k_{20}$</td>
<td>A rate from post-power-stroke to unbound state</td>
<td>20 (s$^{-1}$)</td>
</tr>
<tr>
<td>$F_0$</td>
<td>Constant for force dependence</td>
<td>$5.04 \times 10^{-12}$ (N)</td>
</tr>
<tr>
<td>$E_{pp}$</td>
<td>Free energy bias toward the post-power-stroke state</td>
<td>$-60 \times 10^{-21}$ (J)</td>
</tr>
<tr>
<td>$E_{ext}$</td>
<td>External energy contribution</td>
<td>$-60 \times 10^{-21}$ (J)</td>
</tr>
<tr>
<td>$d$</td>
<td>Step size</td>
<td>$7 \times 10^{-9}$ (m)</td>
</tr>
<tr>
<td>$k_m$</td>
<td>Spring constant of the neck linkers</td>
<td>$1 \times 10^{-3}$ (N/m)</td>
</tr>
</tbody>
</table>
Figure 2.2. (a) Walking ($k_{w,M}$) and (b) unbinding rates ($k_{u,M}$) of motor arms depending on force acting on the arms. They behave as a catch bond, leading to lower $k_{w,M}$ and $k_{u,M}$ with higher applied forces. Unloaded walking velocity is 140 nm/s (= 7 nm $\times k_{w,M}$ at zero force), and stall force beyond which the arms stop walking is 5.7 pN. (All parts were reprinted from [13].)
3. FORCE GENERATION IN CORTICAL CYTOSKELETAL NETWORKS

(Most of this section is adopted from [13])

Living cells utilize mechanical forces for critical biological functions such as cytokinesis, cell migration, and muscle contraction [32]. Actomyosin contractility is responsible for generating tensile forces in muscle, smooth muscle, and non-muscle cells, leading to various morphological changes from subcellular to tissue length scales [33–35]. The cell cortex is a thin network of F-actins, cross-linked by ACPs and decorated by myosin molecular motors, whose local contractility drives the growth of spherical membrane protrusion called blebs and large-scale contractility facilitates the detachment and retraction of cell body during migration [36]. Tight regulation of cortical contractility is crucial for determining and maintaining polarization axis during cell division [37]. The cortex often undergoes dramatic structural remodeling due to F-actin turnover and myosin-mediated contractions, which enables cells to survive in rapidly changing extracellular environments [38]. Thus, understanding how the cell cortex generates forces and structurally remolds is a prerequisite for elucidating the underlying mechanisms of biological processes including migration and division.

A wide variety of in vitro reconstitution experiments have provided insights into the understanding of actomyosin contractility. Initial studies recombined purified F-actins, myosins, and ACPs to identify the minimal prerequisites for contractility and structural reorganization of disorganized actomyosin networks. As a result, it has been established that the dynamics and extent of contractility are regulated by F-actin network architecture [39], as well as the density and interplay of myosin motors and ACPs [40–45]. From these studies, it was found that the contractility arises from a symmetry breaking between tensile and compressive forces via buckling of F-actin [46, 47]. In addition, it has been shown that molecular motors can tune the viscoelastic properties of networks. For example, mechanical stress arising from
molecular motor and ACP activities significantly increases the stiffness of actomyosin networks by more than two orders of magnitude in an ATP-dependent manner [48,49].

Concurrently, various computational and theoretical approaches have been developed to study force generation in actomyosin networks. The origin of force generation has been associated with local deformations of F-actin which result in net tension on networks by breaking symmetry between tensile and compressive forces [50,51]. By contrast, the propensity of motors to reposition toward lower energy configurations can also lead to net tensile stress [52]. The force generation can lead to changes in the structural organization of F-actin networks. At elevated motor density, contractile forces drive the formation of bundles from disordered networks [53]. In the presence of ACPs, stress generated by motors drives initially well-connected networks to a critical state with the formation of clusters and rupture of ACPs, or stabilizes initially floppy networks, enhancing network stiffness [54–56].

However, to date, few computational models have quantitatively described the time-variant contractile behaviors of cortical actomyosin networks or have faithfully captured the detailed geometry and mechanochemistry of myosin thick filaments and the mechanics and kinetics of F-actins and ACPs. Thus, we used the agent-based computational model introduced in the methods section to study force generation and structural remodeling of cortical actomyosin networks. First, the maximum level and sustainability of stress generated by the networks as well as changes in network morphology over a wide range of densities of motors and ACPs were evaluated. We found that motors are able to behave as temporary cross-linkers during initial stress generation, leading to larger maximum stress with higher motor density. However, the generated stress relaxes over time unless there are a sufficient amount of ACPs. We also showed that ACPs enable motors to exert forces close to their maximum potential, significantly enhancing the efficiency and stability of stress generation. In addition, it was demonstrated that the frequency of detachment of ACPs from F-actin has a dramatic impact on the accumulation of network stress and long-term changes in network morphology. These findings are consistent with those that we
found in a previous study using actomyosin bundles with randomly aligned F-actins [24]. Thus, the mechanisms that govern contractility and force generation are robust across diverse F-actin architecture and dimension.

3.1 Methods

3.1.1 Network preassembly

As in previous study [16], cross-linked actomyosin networks are preassembled via self-assembly of actin monomers (G-actin), ACPs, and motors within a thin three-dimensional rectangular domain ($8 \times 8 \times 0.5 \text{ µm}$) with periodic boundary condition (PBC) in x and y directions. Two boundaries located in z direction exert repulsive forces only in the z direction to keep network elements within the domain. During the self-assembly, G-actins are polymerized into F-actins, and the TF structure of motors is formed via nucleation and polymerization of motor backbone segments with their arms binding to F-actins in the absence of walking events. Concurrently, ACPs also bind to F-actins, forming functional cross-links between pairs of F-actins. Then, the PBC is deactivated in the x direction, and F-actins crossing the x boundaries are severed and irreversibly clamped to the x boundaries (Figure 3.1). No F-actins are allowed to be clamped to the x boundaries during simulations. Unless specified, we used the following reference parameter values: initial G-actin concentration ($C_A$) is 25 µM, average F-actin length ($\langle L_f \rangle$) is 1.44 µm, and the molar ratios of motors ($R_M = C_M/C_A$) and ACPs ($R_{ACP} = C_{ACP}/C_A$) are 0.014 and 0.1, respectively. Note that $R_M$ takes all myosin heads into account.

3.1.2 Measurement of force, stress, and elastic moduli

After the network preassembly, it is assumed that the x boundaries behave as an elastic substrate with Youngs modulus, $E$, set to $3 \times 10^4$ Pa (Figure 3.1). Normal stress generated by a network over time is calculated by $\sigma(t) = E\epsilon(t)$ where $\epsilon(t)$ is
Figure 3.1. A thin cortex-like network is clamped to the x boundaries which behave as an elastic substrate with Young's modulus, $E$, set to $3 \times 10^4$ Pa. Periodic boundary condition (PBC) is applied in y direction to simulate an infinitely large network. (Reprinted from [13].)

the normal strain of the elastic substrate in x direction. We calculated the maximum $\sigma_{\text{max}}$ and sustainability ($S = \langle \sigma \rangle / \sigma_{\text{max}}$) of the normal stress, where $\langle \sigma \rangle$ indicates the time average of $\sigma(t)$ between a time point when $\sigma$ is $\sigma_{\text{max}}$ and $t = 100$ s.

We also evaluated microscopic forces acting on motors when $\sigma$ is close to $\sigma_{\text{max}}$, $f_{M}^{\text{max}} = \vec{F}_{S,M4} \cdot \vec{u} / N_h$, and on ACPs, $f_{ACP}^{\text{max}} = \vec{F}_{S,ACP} \cdot \vec{u}$, where $\vec{F}_{S,M4}$ is force acting on the spring governed by $\kappa_{u,M}$, and $\vec{u}$ is a unit vector in the axial direction of an actin segment toward a barbed end. Note that $f_{M}^{\text{max}}$ and $f_{ACP}^{\text{max}}$ are positive when acting toward barbed ends of F-actins. As shown later (Figures 3.6b, 3.8b, 3.9b), $f_{M}^{\text{max}}$ is predominantly positive because $\kappa_{u,M}$ becomes very large if $\vec{F}_{S,M4}$ is directed toward a pointed end. By contrast, $f_{ACP}^{\text{max}}$ shows largely symmetric distribution but is biased slightly toward negative values since ACPs sustain positive $f_{M}^{\text{max}}$.

Frequency-dependent storage ($E'$) and loss moduli ($E''$) are evaluated by measuring stress in response to a small sinusoidal strain (5%) applied to the +x boundary of the domain with the -x boundary fixed in a similar way to that described in a
Phase delay between the applied strain and measured stress is calculated as $\tan^{-1}(E''/E')$.

3.1.3 Evaluation of changes in network morphology

During force generation, actomyosin networks show changes in morphology including the spatial distribution of motors and network mesh size. Spatial redistribution of motors is quantified by calculating the distribution of separation distances between a centroid position of each motor TF and that of its nearest neighbor. Predominance of distances close to zero in the distribution is indicative of motor aggregation because the zero distances originate from accumulation of motors in the same vicinity. Thus, we evaluated a temporal increase in the relative frequency of the smallest separation distance in distribution of the distances between the nearest neighbor motors to estimate time evolution of the motor aggregation in each simulation.

In addition, variations in network mesh size are indirectly estimated by approximating the density distribution of the network [57]. The domain of the network is divided to $400 \times 400$ pixels in x and y directions, and the intensity of each pixel is one if any actin segment is located on the pixel but zero if not. Then, distribution of the distances between the nearest neighbor non-zero pixels within each column (400 pixels) aligned in the y direction is calculated. If a network exhibits a homogeneous morphology, the distribution can be fitted well by an exponentially decreasing function. Smaller exponential decay constant is indicative of larger mesh size. We evaluated a temporal decrease in the decay constant in the distribution to estimate time evolution of network mesh size in each simulation. Note that the z position of actin segments is not reflected for this estimation, which can lead to underestimation of network mesh size. However, a relative change in network mesh size over time can still be captured.
3.2 Results and Discussions

3.2.1 Factors that govern mechanical tension and the viscoelasticity of actomyosin networks

We explored factors that govern the generation of mechanical tension and the viscoelasticity of cortex-like actomyosin networks. First, we probed the time evolution of stress, $\sigma(t)$, and evaluated storage and loss moduli ($E'$ and $E''$) with three different combinations of molar ratios of motors ($R_M$) and ACPs ($R_{ACP}$).

Figure 3.2. Time evolution of stress, $\sigma(t)$, with three combinations of densities of motors ($R_M$) and ACPs ($R_{ACP}$): $R_M = 0.14$ and $R_{ACP} = 0.1$ (green diamonds), $R_M = 0.014$ and $R_{ACP} = 0.1$ (blue circles), and $R_M = 0.014$ and $R_{ACP} = 0.01$ (red triangles). (Reprinted from [13].)

At low motor activity and high cross-linking density ($R_M = 0.014$ and $R_{ACP} = 0.1$), $\sigma$ quickly reaches its maximum, $\sigma_{max} \sim 600$ Pa, and then remains relatively constant (Figure 3.2). The network remodels nominally with only small changes in distribution of mesh size, motors, and forces for 100 s (Figure 3.3a). In this case, constant network morphology is concomitant with constant elasticity. $E'$ shows a very weak power-law dependence on frequency, $f^{0.09}$, while $E''$ is minimum at 1 Hz (Figure
Figure 3.3. Structural reorganization correlates with stiffening and softening of networks. Network morphology and viscoelastic moduli with three combinations of $R_M$ and $R_{ACP}$ used in Figure 3.2 (a, c, e). Distribution of distances between each motor and its closest neighbor and (inset) distribution of mesh size of networks measured at three time points. An increase in the relative frequency of the most left data point in the distribution of distances between the nearest neighbor motors indicates aggregation of motors. Note that distances are much smaller in (e) due to higher $R_M$. Dashed lines in the insets show exponential fits to the distribution of mesh size, and a decrease in the exponential decay constant over time is indicative of an increase in mesh size. (b, d, f) Storage, $E'$, and loss moduli, $E''$, measured at three time ranges. A legend in (b) is shared with (d) and (f). Cyan inverted triangles show $E'$ and $E''$ in the absence of ACP unbinding. (All parts were reprinted from [13].)
Figure 3.4. Viscoelastic properties of networks depending on frequency and time. (a-c) Phase delay, $\tan^{-1}E'/E''$, between applied strain and measured stress measured at three different time ranges with three sets of $R_M$ and $R_{ACP}$. A legend in (a) is shared with (b) and (c). Cyan inverted triangles show the phase delay of cases with the same sets of $R_M$ and $R_{ACP}$ without ACP unbinding. Perfectly elastic and viscous materials exhibit the phase delay of 0° and 90°, respectively. (d) $E''$ at 1 Hz vs stress ($\sigma$) measured at $t = 10-60$ s with three sets of $R_M$ and $R_{ACP}$. Brighter symbols represent early times while darker ones represent later times. (All parts were reprinted from [13].)

3.3b), and the network becomes the most elastic with the smallest phase delay at 1 Hz. (Figure 3.4a). After $\sigma$ reaches $\sigma_{\text{max}}$, $E'$ and $E''$ change minimally over time. By contrast, at low motor activity and low cross-linking density ($R_M = 0.014$ and $R_{ACP} = 0.01$), both $\sigma_{\text{max}}$ and the sustainability, $S$, are reduced (Figure 3.2), and the network morphology drastically changes over time, resulting in very large meshes and severe coalescence of motors (Figure 3.3c). At the end, the stress becomes nearly zero with emergence of separated aggregates. At $\sigma \sim \sigma_{\text{max}}$, $E'$ is much smaller but still shows the
weak power-law dependence at low frequencies (Figure 3.3d) with the minimal phase delay at 3.16 Hz. As $\sigma$ diminishes over time, the network exhibits a large reduction in $E'$ with greater power-law exponent while $E''$ remains relatively constant, resulting in greater phase delay (Figure 3.4b). The rise of $E''$ at low frequencies gradually disappears over time. Thus, network remodeling modulated by ACPs is inversely related to network elasticity and to the ability to sustain mechanical stress. With high motor activity and high cross-linking density ($R_M = 0.14$ and $R_{ACP} = 0.1$), the generated stress is destabilized but shows significantly enhanced $\sigma_{\text{max}}$ (Figure 3.2). In addition, the accumulation of stress is accompanied by gradual coarsening of the network with increasing mesh size and aggregation of motors (Figure 3.3e). $E'$ is very high and nearly independent of frequency, showing a slow decrease over time (Figure 3.3f), and the phase delay becomes minimal at 10-31.6 Hz (Figure 3.4c).

Overall, the elasticity of the network tensed by motor activity is strongly dependent upon the kinetics of ACP unbinding. Without unbinding of ACPs, $E'$ is high with very weak frequency dependence and hardly changes over time, and $E''$ shows a much smaller increase at low frequencies in all the three cases (Figures 3.3b, d, f). This indicates that the temporal decrease in $E$ occurring with ten-fold higher $R_M$ or ten-fold lower $R_{ACP}$ and the existence and variation of the critical frequency are associated with the ACP unbinding.

Interestingly, $E'$ and $E''$ are closely related to $\sigma$. $E'$ at a low frequency (1 Hz) is directly proportional to $\sigma$ at high $\sigma$ but becomes independent of $\sigma$ at low $\sigma$ as found in other study [56] (Figure 3.4d). In the three cases tested above, exponent of the power-law dependence of $E'$ on frequency is shown to be inversely proportional to $\sigma$. $E''$ at high frequencies is relatively the same regardless of $R_M$ and $R_{ACP}$, whereas the critical frequency at which a network becomes the most elastic tends to be higher when $\sigma$ is not sustained well (i.e. low $S$). All these are consistent with characteristics of frequency-dependent shear moduli, $G'$ and $G''$, in a previous experiment [49]. Therefore, $E'$ and $E''$ are roughly predictable if $\sigma_{\text{max}}$ and $S$ are known.
3.2.2 Densities of motors and ACPs govern stress generation and network morphology

To find a quantitative relationship between the molar ratios and the generated stress, we systematically evaluated $\sigma_{\text{max}}$ and $S$ over a wide range of $R_M$ and $R_{ACP}$ (Figures 3.5a,b), and visualized network morphology and force distribution when $\sigma$ reaches $\sigma_{\text{max}}$ (Figures 3.5c, d). Overall, $\sigma_{\text{max}}$ increases with proportionality to both $R_M$ and $R_{ACP}$, and $S$ tends to be greater with smaller $R_M$ and larger $R_{ACP}$. A similar level of $\sigma_{\text{max}}$ can be acquired with much fewer motors if a sufficient number of ACPs exist in a network, indicative of complementary effects of motors and ACPs on $\sigma_{\text{max}}$ (Figure 3.5a). However, $S$ of cases with similar $\sigma_{\text{max}}$ can be quite different (Figure 3.5b), so a relationship between $\sigma_{\text{max}}$ and $S$ seems unclear. For better understanding about the relationship, we focused on how either $R_M$ or $R_{ACP}$ affects $\sigma_{\text{max}}$ and $S$ with the other molar ratio fixed.

First, it was found that adding more motors at a constant level of $R_{ACP}$ tends to increase $\sigma_{\text{max}}$ but reduce $S$ with facilitation of network remodeling (Figure 3.6). For example, at $R_{ACP} = 0.1$ and low $R_M$, $\sigma_{\text{max}}$ increases in proportion to low $R_M$ following $\sigma_{\text{max}} \sim R_M^{0.6}$ with $S$ close to 1 (Figure 3.6a), and network morphology (i.e. mesh size and motor aggregation) hardly changes after $\sigma$ reaches $\sigma_{\text{max}}$ (Figures 3.6c, d). However, above $R_M \sim 0.06$, $\sigma_{\text{max}}$ slightly deviates from the power-law dependence, and $S$ rapidly drops with a decrease in $f_M^{\text{max}}$ and an increase in $f_{ACP}^{\text{max}}$ (Figure 3.6b) as well as large variations in the network morphology over time. $\sigma_{\text{max}} \sim R_M^{0.6}$ at lower $R_M$ and the variations at higher $R_M$ (deviation from the power-law dependence and sharp drop of $S$) are consistent at lower $R_{ACP}$ (Figures 3.7a, b). Since $R_{ACP}$ is fixed, a greater number of motors result in larger force acting on each ACP, which destabilizes bonds between ACPs and F-actins by increasing $k_{u,ACP}$ due to the force dependence described in Equation (2.8). The destabilization prevents motors from exerting their maximum potential forces, $f_{M}^{\text{stall}}$, and leads to lower $S$. It also facilitates large-scale remodeling of otherwise stable networks after $\sigma \sim \sigma_{\text{max}}$. 
Figure 3.5. F-actin cross-linking and motor activity synergistically increase the magnitude and sustainability of mechanical stress. Effects of $R_M$ and $R_{ACP}$ on (a, b) the maximum, max, and sustainability of the stress, S, and on (c, d) the morphology of networks at $\sigma \sim \sigma_{\text{max}}$. In (c), F-actins, ACPs, and motors are indicated by cyan, yellow, and red, respectively. In (d), level of forces exerted on each constituent is represented by the color scaling. (All parts were reprinted from [13].)

Second, we found that addition of ACPs at a fixed level of $R_M$ significantly increases $\sigma_{\text{max}}$ and $S$ by helping motors to exert their stall forces but suppresses network remodeling (Figure 3.8). For example, at $R_M = 0.014$, $\sigma_{\text{max}}$ rapidly increases from $R_{ACP} = 0.001$ and reaches a plateau, $\sim 600$ Pa, at $R_{ACP} \sim 0.05$ (Figure 3.8a). Existence of the plateau is attributed to the limited number of motors and their finite stall force as can be seen in various plateau levels depending on $R_M$ (Figure 3.7). At $R_M =$
Figure 3.6. Motor activity promotes stress generation and network remodeling but is antagonistic to stress sustainability. At $R_{ACP} = 0.1$, influences of $R_M$ on (a) $\sigma_{max}$ (red circles) and $S$ (blue triangles), (b) distribution of forces acting on motors ($f_{M}^{max}$) and ACPs ($f_{ACP}^{max}$), (c) a decrease in the decay constant of an exponential fit to the distribution of mesh size at $\sigma \sim \sigma_{max}$ or $t = 100$ s, compared to that of the fit at $t = 0$ s, and (d) an increase in the relative frequency of the most left data point in the distribution of distances between the nearest neighbor motors at $\sigma \sim \sigma_{max}$ or $t = 100$ s, compared to that at $t = 0$ s. In (c), a larger decrease in the decay constant represents a larger increase in network mesh size, and in (d), a larger increase in the relative frequency is indicative of more severe motor aggregation. (All parts were reprinted from [13].)

0.014, $S$ shows a sharp increase from $R_{ACP} \sim 0.005$ and becomes nearly one at $R_{ACP} \sim 0.1$. As the fixed level of $R_M$ increases, $S$ begins to abruptly increase from higher $R_{ACP}$ (Figure 3.7d). At $R_M = 0.014$ and $R_{ACP} > 0.1$, the network morphology varies negligibly after $\sigma$ reaches $\sigma_{max}$ (Figures 3.8c, d). As above, with low $R_{ACP}$, motors are incapable of exerting forces close to $f_{M}^{max}$ while $f_{ACP}^{max}$ is large (Figure 3.8b). However,
as $R_{ACP}$ increases, $f_M^{\text{max}}$ rises to a level close to $f_M^{\text{stall}}$, whereas $f_{ACP}^{\text{max}}$ diminishes. Thus, with more ACPs, the force dependence of ACP unbinding is not likely to play an important role, stabilizing the bonds between ACPs and F-actins with higher $\sigma_{\text{max}}$ and $S$ and with minimal network remodeling.
Figure 3.8. F-actin cross-linking increases stress generation and sustainability concomitantly but is antagonistic to network remodeling. At $R_M = 0.014$, impact of $R_{ACP}$ on (a) $\sigma_{\text{max}}$ (red circles) and $S$ (blue triangles), (b) distribution of $f_{\text{M}}^{\text{max}}$ and $f_{\text{ACP}}^{\text{max}}$, (c) a decrease in the decay constant of an exponential fit to the distribution of mesh size, and (d) an increase in the relative frequency of the most left data point in the distribution of distances between the nearest neighbor motors at $\sigma \sim \sigma_{\text{max}}$ or $t = 100 \text{ s}$, compared to those at $t = 0 \text{ s}$. (All parts were reprinted from [13].)

3.2.3 Stability of ACPs determines long-time evolution of generated stress and morphology

$\sigma_{\text{max}}$ is proportional to both molar ratios since more motors provide greater force, and more ACPs can help the motors to exert their stall forces. For determining $\sigma_{\text{max}}$, long-term stability of the bonds between ACPs and F-actins is not significant since it takes a relatively short time for $\sigma$ to reach $\sigma_{\text{max}}$, and motors can contribute to enhancing connectivity temporarily as can be clearly seen in a difference of morphology.
and force distribution between a case with $R_{ACP} = 0.01$ and $R_M = 0.008$ and that with $R_{ACP} = 0.01$ and $R_M = 0.08$ (Figures 3.5c, d) when $\sigma$ reaches $\sigma_{\text{max}}$. Network remodeling at this early stage occurs minimally via local deformation of F-actins without large-scale network remodeling induced by numerous unbinding events of ACPs (Figures 3.6c, d and Figures 3.8c, d). By contrast, $S$ is proportional to $R_{ACP}$ but inversely proportional to $R_M$ because the stability of the bonds is very crucial for high $S$. The long-term stability is highly affected by whether or not the force dependence of ACP unbinding is activated by large force exerted on ACPs. The magnitude of the force acting on ACPs is likely to be roughly proportional to the ratio of $R_M$ to $R_{ACP}$, which explains the relation between $S$ and the two molar ratios. Substantial network remodeling may emerge with large increases in mesh size and motor aggregation if the force-induced destabilization of ACP bonds occurs. In such a case, major structural reorganization of networks occurs during relaxation of generated stress, not during stress generation.

### 3.2.4 Kinetics of ACPs differentially regulates stress generation and network architecture

As an increase in $k_{u,ACP}$ caused by the force sensitivity has the large effects, we found that an increase in the zero-force unbinding rate coefficient, $k_{u,ACP}^0$, reduces $\sigma_{\text{max}}$ and $S$ but promotes the network remodeling (Figure 3.9). $\sigma_{\text{max}}$ and $S$ are less sensitive to $k_{u,ACP}^0$ at $k_{u,ACP}^0 < k_{u,ACP}^{0^*}$, but become inversely proportional to $k_{u,ACP}^0$ at $k_{u,ACP}^0 > k_{u,ACP}^{0^*}$ (Figure 3.9a). The inverse proportionality of $\sigma_{\text{max}}$ and $S$ to $k_{u,ACP}^0$ hardly changes despite a variation in $R_M$ or $R_{ACP}$ (Figure 3.10). Dependence of $\sigma_{\text{max}}$, $S$, and the network morphology on $k_{u,ACP}^0$ (Figures 3.9a, c, d) seems opposite to that on $R_{ACP}$ (Figures 3.8a, c, d) because increases in $k_{u,ACP}^0$ and $R_{ACP}$ have opposite effects on the molar ratio of ACPs in the active state, $R_{ACP}^{\text{active}}$ (i.e. the number of ACPs bound to pairs of F-actins at a dynamic equilibrium). If a decrease in $R_{ACP}^{\text{active}}$ is a sole outcome induced by an increase in $k_{u,ACP}^0$, $\sigma_{\text{max}}$ and $S$ should be similar between
Figure 3.9. ACP unbinding decreases stress accumulation and promotes network remodeling. At the fixed levels of $R_M = 0.014$ and $R_{ACP} = 0.1$, effects of zero-force unbinding rate coefficient of ACPs ($k_{0u,ACP}$) on (a) $\sigma_{\text{max}}$ (red circles) and $S$ (blue triangles), (b) distribution of $f_{\text{max}}^M$ and $f_{\text{max}}^{ACP}$, (c) a decrease in the decay constant of an exponential fit to the distribution of mesh size, and (d) an increase in the relative frequency of the most left data point in the distribution of distances between the nearest neighbor motors at $\sigma \sim \sigma_{\text{max}}$ or $t = 100$ s, compared to those at $t = 0$ s. In (b), values in the legend show $k_{0u,ACP}^*/k_{0u,ACP}$ in each case. (All parts were reprinted from [13].)

cases with the same $R_{ACP}^{\text{active}}$, regardless of how $R_{ACP}^{\text{active}}$ is changed. In Figures 3.11a, b, $\sigma_{\text{max}}$ and $S$ of cases where $R_{ACP}$ is varied (Figure 3.8a) and of those where $k_{u,ACP}^0$ is changed (Figure 3.9a) with respect to $R_{ACP}^{\text{active}}$ are shown. A large difference in $\sigma_{\text{max}}$ and $S$ between two groups at each $R_{ACP}^{\text{active}}$ was observed, implying that subsequent binding and unbinding of ACPs effectively dissipate elastic energy built by motors rather than merely reduce $R_{ACP}^{\text{active}}$. Indeed, the effect of increasing $k_{u,ACP}^0$ on $f_{ACP}^{\text{max}}$ (Figure 3.9b) is opposite to that of decreasing $R_{ACP}$ (Figure 3.8b) although both changes reduce
Figure 3.10. Importance and effects of $k_{u,ACP}^0/k_{u,ACP}^{0*}$ on (a, c) $\sigma_{\text{max}}$ and (b, d) $S$ under the reference condition (red circles) or with a change in a single parameter value (other symbols shown in the legend). Note that the legend in (a) is applied to (b), and the legend in (c) is shared with (d). (All parts were reprinted from [13].)

$R_{\text{ACP}}^{\text{active}}$. Higher $k_{u,ACP}^0$ would allow ACPs to sustain forces only for a short period before ACPs lose the forces due to unbinding, which deteriorates stress generation (low $\sigma_{\text{max}}$) and maintenance (low $S$) but facilitates the network remodeling.
Figure 3.11. Increased ACP unbinding disturbs stress accumulation effectively rather than merely changes the molar ratio of ACPs in the active state ($R_{ACP}^{active}$) at a dynamic equilibrium. Effects of $R_{ACP}^{active}$ on (a) $\sigma_{max}$ and (b) $S$. $R_{ACP}^{active}$ is altered by either varying $R_{ACP}$ (red circles, data from Figure 3.8) or $k_{u,ACP}^0/k_{u,ACP}^*$ (blue triangles, data from Figure 3.9). $R_{ACP}^{active}$ is measured for 1 s before $\sigma(t)$ reaches $\sigma_{max}$, and dashed lines indicate $R_{ACP}^{active} = 0.1$. (All parts were reprinted from [13].)

3.2.5 ACPs help motors to generate the maximum force by inducing friction between F-actins

Dependence of $\sigma_{max}$ and $S$ on $R_M$, $R_{ACP}$, and $k_{u,ACP}^0$ can be explained by local interactions between motors and ACPs. Motors tend to pull pairs of F-actins in opposite directions, which is capable of developing a mechanical force whose magnitude varies depending on how freely F-actins are displaced. If the F-actins are stably anchored to other F-actins by ACPs or clamped to boundaries, the motors will easily reach their stall force because force can quickly be built by their walking motion. By contrast, if the F-actins are unstably anchored or free to move, the F-actins can glide across motors without generation of large forces. Considering a small percentage of the clamped F-actins, the transient connection between F-actins via ACPs plays a critical role for helping motors to produce their maximum stall forces. The transience makes ACPs behave as a molecular clutch that transmits forces between pairs of F-actins via effective friction that is governed by $k_{u,ACP}^0$ and how much force is acting on each ACP at given values of $R_M$ and $R_{ACP}$. Long-term changes in network
morphology accompanied by large meshes and severe motor aggregation may or may not occur, depending on whether or not the transient connection becomes unstable by the force acting on ACPs.

3.2.6 Force-induced destabilization of ACPs also affects frequency-dependent viscoelastic moduli

Initially, using three sets of $R_M$ and $R_{ACP}$, we showed that the critical frequency where a network becomes the most elastic (i.e. the lowest phase delay) can shift to higher frequencies by either increasing $R_M$ or decreasing $R_{ACP}$, and that the local minimum of $E''$ disappears if ACPs do not unbind (Figures 3.3b, d, f). In other studies, it has been shown that the critical frequency represents the transition point below which ACPs begin to behave as transient cross-linkers and therefore elevate loss modulus, and that the critical frequency can vary or disappear by tuning unbinding rates of ACPs [58,59]. The shift of the critical frequency observed in our results can be explained by force-induced acceleration of ACP unbinding. As the ratio of $R_M$ to $R_{ACP}$ becomes greater by ten-fold higher $R_M$ or ten-fold lower $R_{ACP}$, $k_{u,ACP}^0$ dramatically increases due to large forces acting on ACPs, resulting in the shift of the critical frequency to higher frequencies. This is why the increase in the critical frequency is associated with low $S$ that is also caused by the force-induced destabilization.
4. STRESS RELAXATION IN PASSIVE CYTOSKELETAL NETWORKS

(Most of this section is adopted from [14])

Living cells navigating extracellular environments are continually subjected to a wide spectrum of mechanical forces. The response of the actin cytoskeleton to these forces mediates cellular processes including migration, division, and mechanotransduction [2]. Reconstituted networks consisting of F-actins cross-linked by actin cross-linking proteins (ACPs) have provided a simplified framework for probing the basic mechanisms of rheological responses of the actin cytoskeleton since the complicated effects of cell signaling networks and active regulation of actin-associated proteins are excluded [60]. A representative response of cross-linked F-actin networks to increasing shear strain is strain-stiffening above a critical strain, which has been observed in various reconstituted experiments [61]. Computational studies have further found that strain-stiffening is attributed to a transition from a bending-dominated non-affine deformation regime at low strains to a stretching-dominated affine deformation regime at high strains [62–64]. At high strains, a small fraction of F-actins bearing extensional forces, named supportive framework, support most of the stress (Figure 4.1) [15, 65]. Emergence of negative normal stress during the strain-stiffening also shows that F-actins in the supportive framework are stretched at high strains [66]. Interestingly, a computational study demonstrated that some of F-actins located outside the supportive framework are buckled at high strains [67]. If strain is further increased, the networks exhibit stress relaxation to an extent depending on strain rates [27, 68], attributed mostly to force-dependent unbinding of ACPs from F-actin as shown in computational studies [17, 69]. The cross-linked networks also show gradual stress relaxation over cycles when subjected to large oscillatory strains; stress at peak strains keeps decreasing over cycles [70–72]. Although it was speculated that
the cyclic stress relaxation might be involved with breakage of F-actin, its origin has remained elusive.

It has been observed that F-actins are severed and fragmented into shorter filaments due to molecular motor activity and thermal fluctuation [46, 73, 74]. An increase in local bending of F-actins enhances the probability of severing by destabilizing bonds between adjacent actin subunits [75]. This implies that the bending of F-actins in the supportive framework at low strains [15, 64] and buckling of F-actins outside the supportive framework at high strains may promote more frequent severing events. Severing can impair structural integrity of networks more than unbinding of ACPs that can be followed by rebinding on intact F-actins [17]. Thus, the F-actin severing might be the factor causing the stress relaxation over cycles in response to oscillatory strains. Nevertheless, it is still unclear how the F-actin severing can give rise to the cyclic stress relaxation of cross-linked networks.

In this study, using the computational model introduced in the methods section, we explored effects of F-actin severing on the cyclic stress relaxation of three-dimensional cross-linked actin networks. We subjected cross-linked F-actin networks to various cyclic shear strains and monitored the buildup and relaxation of shear stress with and without severing of F-actin. We found that severing of F-actins is mainly caused by buckling at high strains and leads to pronounced stress relaxation especially for highly cross-linked networks consisting of long F-actins. We also compared and contrasted the impacts of F-actin severing to ACP unbinding to which stress relaxation has been predominantly attributed [17], and further demonstrated their cooperative effects on the mechanical response of F-actin networks.

4.1 Methods

4.1.1 Network preassembly

Cross-linked F-actin networks are formed via self-assembly of actin monomers and ACPs within a three-dimensional rectangular domain with a periodic boundary
Figure 4.1. Schematic diagrams representing networks composed of F-actins (cyan, red, blue) and actin cross-linking proteins (ACPs, orange) within a cubical domain before (left) and after application of shear strain (right). A portion of F-actins oriented diagonally (red) experience extensional forces and contribute to the buildup of large shear stress as a supportive framework, whereas the other F-actins oriented perpendicularly to the F-actins in the supportive framework (blue) are compressed and prone to buckle. For simplicity, it is assumed that the red F-actins are not connected to the blue ones in the diagrams although they are inter-connected via ACPs. A periodic boundary condition is applied in x and y directions to simulate infinitely large networks. (Reprinted from [14].)

condition, as in the previous studies [15,17]. The dimension of the domain is $3 \times 3 \times 3 \, \mu m$ for most simulations while a larger domain ($6 \times 6 \times 6 \, \mu m$) is used for Figure 4.8 to test effects of average length of F-actin, $\langle L_f \rangle$, over a wide range. During the network formation, the actin monomers assemble to form F-actins, and ACPs bind to F-actins to form functional cross-links between pairs of F-actins. After the network formation, F-actins at the upper and lower boundaries are severed and clamped, and the periodic boundary condition is deactivated in z-direction (Figure 4.1). Then, cyclic shear strain with a constant strain rate, $\dot{\gamma}$, is imposed. Actin segments located within 140 nm from the bottom surface are fixed, whereas those within 140 nm from the top surface are displaced following a strain profile. Resultant stress exerted by a network is measured by summing the x component of forces acting on ends of the F-actins clamped to the top surface and then dividing the sum by the area of the top surface as in our previous work [17].
4.1.2 Severing of F-actin

Severing of F-actin is simulated by breaking a chain between two adjacent points on the F-actin, which corresponds to disappearance of one actin segment. To determine the rate of stochastic severing events, the sum of two adjacent bending angles, $\theta_0 = \theta_1 + \theta_2$, on each actin segment is calculated (Figure 4.2a). Since there is no model that describes dependence of the severing rate on the bending angle, we determined a relationship between the severing rate ($k_{\text{sev}}$) and the bending angle ($\theta_b$) in an empirical fashion. An in vitro experiment showed that the severing can take place at large bending angles due to thermal fluctuation [7]. We measured $\theta_b$ of thermally fluctuating F-actins in our model and found exponentially decreasing distribution (Figure 4.2b, inset). Thus, $k_{\text{sev}}$ needs to be extremely sensitive to an increase in $\theta_b$ in order to have severing events only at large $\theta_b$. Thus, we assume that $k_{\text{sev}}$ exponentially increases as $\theta_b$ increases:

$$k_{\text{sev}} = k^0_{\text{sev}} \exp \left( \frac{\theta_b}{\lambda_{\text{sev}}} \right) \quad (4.1)$$

where $k^0_{\text{sev}}$ is a zero-angle severing rate coefficient, and $\lambda_{\text{sev}}$ defines sensitivity to $\theta_b$. The values of $k^0_{\text{sev}}$ and $\lambda_{\text{sev}}$ were determined by comparing the distribution of $\theta_b$ where severing occurred in the simulations with the experimental observation, $57 \pm 9^\circ$ (Figure 4.2b) [7]. Note that $\theta_b$ did not exceed $68^\circ$ even in the long-time simulations with the persistence length of 9$\mu$m. Severing angles greater than $68^\circ$ measured in the in vitro experiment may have originated from overestimation of angles in image analysis or local fixation of F-actins on a surface in the experiment. Otherwise, the discretization of F-actin to serially connected cylindrical segments could prevent $\theta_b$ from increasing above $68^\circ$. Nevertheless, Equation (4.1) results in a good fit between simulations and experiment $\theta < 68^\circ$.

Note that Equation (4.1) is merely an approximation for the dependence of $k_{\text{sev}}$ on $\theta_b$ obtained from comparison between computational and experimental results. The value of $\lambda_{\text{sev}}$ would differ if we use only one bending angle for Equation (4.1) (e.g. $\theta_b = \theta_1$) or if a way of the discretization of F-actin to cylinders is changed.
4.2 Results and Discussions

4.2.1 Buckling of F-actin leads to stress relaxation

Initially, we measured shear stress of networks in response to bi-directional cyclic strain with amplitude of 50 % that is large enough to induce non-linear rheological
behaviors [27, 61] (Figure 4.3a). Note that unless specified, unbinding of ACPs was prevented to isolate effects of F-actin severing on stress relaxation. Stress level hardly changes over cycles in the absence of severing. By contrast, stress is substantially relaxed after the first cycle in the presence of severing, which is quite similar to the observation from in vitro experiments where cross-linked F-actin networks were subjected to sinusoidal strains [70–72]. The degree of stress relaxation depends on the severing rate of F-actins (Figure 4.4), which implies that F-actin severing leads to the significant stress relaxation. Since the severing was modeled as a stochastic event (Equation (4.1)), the stress relaxation is also dependent on a strain rate (Figures 4.5a, b).

Previous computational studies have shown that during the shearing deformation, F-actins experience bending at low strains with non-affine network deformation [15, 64], whereas at high strains, a portion of F-actins are buckled due to compression [67] as depicted in Figure 4.1. The bending at low strains and buckling at high strains have potential to facilitate severing of F-actins since both of them contribute to an increase in bending angles. To uncover the actual origin of the F-actin severing that caused stress relaxation over cycles, we quantified stress relaxation of networks subjected to bi-directional strain with diverse amplitudes by estimating a percentage drop between stress at the first maximal positive strain ($\sigma_1$) and stress at the second maximal positive strain ($\sigma_2$).

$$\text{Drop in stress} = \frac{\sigma_1 - \sigma_2}{\sigma_1} \times 100 \quad (4.2)$$

The stress relaxation is more apparent in networks with larger amplitudes (Figure 4.3b), indicating that buckling occurring at high strains is the origin of the F-actin severing. Indeed, in the initial simulation with severing and amplitude of 50 % shown in Figure 4.3a, severing occurred more frequently when strain was approaching its maximum and minimum, 50 % (Figure 4.3b, inset). Proportionality of the extent of the cyclic stress relaxation to the amplitude of strain is consistent with the experi-
Figure 4.3. F-actins located outside a supportive framework are severed at high strains, leading to stress relaxation. (a) Stress measured from networks with severing (blue triangles) or without severing (red circles) in response to cyclic shear strain (black). Stress relaxation is pronounced only in case with severing. (b) A percentage drop in stress (Equation (4.2)) measured from networks with severing. Stress relaxation is more significant when networks are subjected to bi-directional cyclic strain with larger amplitudes (red circles). By contrast, networks exhibit negligible stress relaxation in response to uni-directional strain, regardless of amplitude (blue triangles). (inset) Frequency of severing events measured from the case in (a). F-actins are severed more when strain is approaching its peaks. (c) Orientation of actin segments severed at $t < 10$ s under different levels of strain. Frequencies are normalized by the largest frequency. Most of the severed actin segments are oriented near $135^\circ$ that is perpendicular to the orientation of a supportive framework ($\sim 45^\circ$). (inset) Orientation of severed actin segments is measured relative to the positive x-axis on the x-z plane. (d) Stress measured from a network with severing (red circles) in response to uni-directional cyclic shear strain (blue). Stress relaxation is negligible although F-actins are severable. (All parts were reprinted from [14].)
Figure 4.4. Cyclic stress relaxation is governed by the F-actin severing rate. (a) Stress measured in networks with $k_{0\text{sev}} = 10^{-17}s^{-1}$ and $\lambda_{\text{sev}} = 1.6 \text{ deg}$ (red circles), $k_{0\text{sev}} = 10^{-21}s^{-1}$ and $\lambda_{\text{sev}} = 1.6 \text{ deg}$ (blue triangles), and $k_{0\text{sev}} = 10^{-17}s^{-1}$ and $\lambda_{\text{sev}} = 1.8 \text{ deg}$ (green inverted triangles) in response to cyclic shear strain (black). Stress relaxation is slower with lower $k_{0\text{sev}}$ and higher $\lambda_{\text{sev}}$. (b, c) A percentage drop in stress between the first and second maximal positive strains, depending on (b) $k_{0\text{sev}}$ and (c) $\lambda_{\text{sev}}$. Interestingly, the stress drop is maximal at $\lambda_{\text{sev}} = 1.6 \text{ deg}$ because larger $\lambda_{\text{sev}}$ allows for F-actin severing only at very large angles, whereas smaller leads too many severing events at small angles even inside the supportive framework. In all cases, $R_{\text{ACP}}$ is 0.032. (All parts were reprinted from [14].)
Figure 4.5. Strain rate ($\dot{\gamma}$) affects stress relaxation. (a, b) Networks with severing only at $R_{ACP} = 0.032$ (a) Stress with $\dot{\gamma} = 0.05 \, s^{-1}$ (red circles) and $2 \, s^{-1}$ (blue triangles) in response to cyclic shear strain (black). (b) A percentage drop in stress between the first and second maximal positive strains, depending on $\dot{\gamma}$. (c, d) Networks with severing and ACP unbinding at $R_{ACP} = 0.1$. (c) Stress with $\dot{\gamma} = 0.05 \, s^{-1}$ (red circles), $0.2 \, s^{-1}$ (blue triangles), and $\dot{\gamma} = 0.4 \, s^{-1}$ (green inverted triangles) in response to cyclic shear strain (black). (d) A percentage drop in stress due to severing (a percentage drop in stress between the first and second maximal positive strains, red circles) and due to ACP unbinding (a percentage drop in stress at first maximal positive strain compared to that in a network without ACP unbinding and severing). (All parts were reprinted from [14].)

itive x axis that roughly corresponds to orientation of F-actins prone to buckling at high strains (Figure 4.1). All these observations support that buckling at high strains induces the severing events, not bending at low strains.
Severing of F-actins facilitated by buckling of F-actin oriented at \( \sim 135^\circ \) is likely to take place outside the supportive framework that contributes to the buildup of large shear stress during strain-stiffening via extension of F-actins oriented at \( \sim 45^\circ \) (Figure 4.1). Then, F-actin severing would not lead to notable stress relaxation during the advance of current strain-stiffening process because the supportive framework would remain almost intact. To confirm it, we subjected networks with severing to uni-directional cyclic strain with amplitude of 50 % (Figure 4.3d). As expected, shear stress was negligibly relaxed over cycles regardless of strain amplitude (Figure 4.3b), compared to the cases with bi-directional cyclic strain.

Figure 4.6 summarizes how buckling and severing of F-actins give rise to stress relaxation in cross-linked networks. F-actins oriented at \( \sim 45^\circ \) and \( \sim 135^\circ \) alternatively become the supportive framework and buckled filaments depending on direction of current strain. Without severing, both groups conserve their structural integrity, resulting in negligible stress relaxation over cycles (Figure 4.6, top row). However, in networks subjected to bi-directional strain with severing, a fraction of the F-actins are severed due to buckling, leading to stress relaxation at strains with an opposite sign (Figure 4.6, middle row). Thus, relaxation of stress at the second maximal strain compared to that at the first maximal strain shown in Figures 4.3a, b was induced by severing of F-actins occurring at negative strains between 20 s and 40 s. By contrast, in response to uni-directional strain, stress relaxation is negligible although F-actins are severed over time (Figure 4.6, bottom row).

4.2.2 In networks with longer F-actins and higher cross-linking density, stress relaxation is more pronounced

It is expected that severing of F-actins caused by buckling would occur to a different extent, depending on conditions. To identify conditions in which severing-induced stress relaxation plays an important role, we quantified the buildup and relaxation of stress exerted by networks under 20 combinations of average filament length \( \langle L_f \rangle \)
Figure 4.6. Severing of F-actins at high strains is attributed to buckling, resulting in stress relaxation at strain with an opposite sign. Schematic diagrams show how F-actins are deformed and severed at the beginning ($t = 0$ s) and peak strains ($t = 10, 30, \text{ and } 50$ s) in networks subjected to cyclic strain under three conditions: with bi-directional strain and no severing (first row); with bi-directional strain and severing (second row); with uni-directional strain and severing (third row). At positive strains, F-actins oriented at $\sim 45^\circ$ (red) experience extensional forces and constitute a supportive framework, whereas those oriented at $\sim 135^\circ$ (blue) are compressed and thus buckled. At negative strains, their behaviors are opposite. Severing occurs in a fraction of buckled F-actins in the cases at the second and third rows as indicated by cyan arrows. In the case with bi-directional strain and severing, a portion of red F-actins is severed at negative strains, leading to stress relaxation at $50$ s while other cases show negligible stress relaxation. (Reprinted from [14].)

and cross-linking density ($R_{ACP}$) in response to bi-directional strain with amplitude of 50 %. Shear stress measured at the first maximal strain increases in proportion to both $\langle L_f \rangle$ and $R_{ACP}$ (Figure 4.7a). Although the explored ranges of $\langle L_f \rangle$ and $R_{ACP}$ are not wide, the largest stress is greater than the smallest one by more than three orders of magnitude, indicative of very high sensitivity of the shear stress to changes in $\langle L_f \rangle$ and $R_{ACP}$. The percentage drop in stress (Equation (4.2)) shows a roughly similar dependence; stress tends to be relaxed more in networks with highly cross-linked long F-actins (Figure 4.7b). However, interestingly, stress relaxation was negligible in networks with higher $\langle L_f \rangle$ and lower $R_{ACP}$ (top-left corner) although stress significantly
Figure 4.7. Stress relaxation induced by severing is more pronounced in networks with longer F-actins and higher cross-linking density ($R_{ACP}$). (a) Stress measured from networks at the first maximal strain with various $R_{ACP}$ and average filament length ($\langle L_f \rangle$) in response to bi-directional strain with amplitude of 50%. The stress is very sensitive to changes in both $\langle L_f \rangle$ and $R_{ACP}$. (b) A percentage drop in stress (Equation (4.2)). Stress relaxation tends to be more severe in networks with higher $R_{ACP}$ and $\langle L_f \rangle$. Note that stress relaxation is negligible at the top-left corner despite large stress because buckling of F-actins hardly results in severing under this condition. (c) Frequency of severing events. Dependence of the frequency on $R_{ACP}$ and $\langle L_f \rangle$ is analogous to that of the percentage drop in (b), implying that severing is responsible for the stress relaxation. (d) Distribution of distances between active ACPs (i.e. functional cross-linking points) measured in four cases corresponding to four corners in (a)-(c). The cross-linking distance shows exponentially decreasing distributions and is highly affected by $R_{ACP}$. (All parts were reprinted from [14].)

dropped in networks with lower $\langle L_f \rangle$ and higher $R_{ACP}$ (bottom-right corner) that showed a similar stress level in Figure 4.7a. Frequency of severing events measured at $t < 10$ s also demonstrates that severing hardly occurred in the networks with higher $\langle L_f \rangle$ and lower $R_{ACP}$ (Figure 4.7c). This is interesting because under such a condition, networks exert relatively high stress and have long cross-linking distances
(Figure 4.7d), which makes F-actins more susceptible to buckling as predicted by Euler beam theory:

\[ F_{\text{buckle}} \sim \frac{\pi^2 K_{b,A} r_{0,A}}{L_c^2} \]  \hspace{1cm} (4.3)

where \( F_{\text{buckle}} \) is a minimum force required for buckling, and \( L_c \) is a cross-linking distance. Note that buckling can lead to severing only when the curvature induced by the buckling is high enough. Buckling with long cross-linking distances (lower \( R_{ACP} \)) results in small curvature (i.e. a large radius of curvature), causing few severing events and negligible stress relaxation. As \( R_{ACP} \) increases, the shear stress increases much faster (Figure 4.7a) than \( F_{\text{buckle}} (L_c^{-2}) \), so buckling occur even at high \( R_{ACP} \). Buckling of F-actins confined via many cross-linking points results in large curvature, facilitating F-actin severing. An increase in \( \langle L_f \rangle \) highly enhances the shear stress (Figure 4.7a), but \( F_{\text{buckle}} \) does not significantly vary because \( L_c \) remains relatively constant under constant actin concentration and \( R_{ACP} \), leading to more F-actin severing events and larger stress relaxation up to \( \sim 70\% \) (Figure 4.8). Thus, severing-induced stress relaxation is more significant in networks with longer F-actins and more ACPs.

### 4.2.3 Severing and ACP unbinding facilitate distinct modes of stress relaxation

In the results presented above, we have ignored the force-dependent unbinding of ACPs from F-actins which has been believed to be a main source of stress relaxation during the strain-stiffening [17]. Indeed, a network only with ACP unbinding shows smaller stress at the first maximal strain, but stress relaxation over cycles is not substantial (Figure 4.9a). It was previously shown that this stress relaxation is mainly attributed to significant unbinding events of ACPs that bear high loads [17]. One may speculate that F-actin severing is less likely to occur in networks with ACP unbinding since reversible cross-linking points might provide F-actins with more freedom to move and rotate. However, it was not the case. A network in the presence of both
Figure 4.8. Average length of F-actins ($\langle L_f \rangle$) has a large effect on cyclic stress relaxation. (a) Stress with $\langle L_f \rangle = 2.14 \, \mu m$ (red circles), $2.73 \, \mu m$ (blue triangles), and $3.62 \, \mu m$ (green inverted triangles) in response to cyclic shear strain (black). (b) A percentage drop in stress between the first and second maximal positive strains, depending on $\langle L_f \rangle$. Note that data for $\langle L_f \rangle$ smaller than $2 \, \mu m$ were obtained in the small domain ($3 \times 3 \times 3 \mu m$), whereas the rest of them were obtained in the large domains ($6 \times 6 \times 6 \mu m$). $R_{ACP}$ is 0.01 in all cases. (All parts were reprinted from [14].)

ACP unbinding and F-actin severing shows a pattern of stress relaxation over cycles that is very similar to that of a network only with F-actin severing (Figure 4.9a). It implies that F-actins were still severed at high strains due to buckling even with ACP unbinding. Since a force required for buckling of F-actins (Equation (4.3)) is not large enough to substantially increase the unbinding rate of ACPs (Equation (2.8)), F-actin buckling can precede significant ACP unbinding events. For example, $F_{buckle}$ for $L_c = 300 \, \text{nm}$ is only 4.1 pN, which increases ACP unbinding rate only 1.1-fold with respect to its reference rate, $k_{u,ACP} = 1.1k_{0,ACP}$. To further understand where ACP unbinding and F-actin severing took place in the network, we evaluated orientations of actin segments from which ACPs unbound with high loads ($|\vec{F}_{s,ACP}| \geq 8 \, \text{pN}$) and at which F-actins were severed. Interestingly, the two events occurred on actin segments oriented orthogonally; while actin segments oriented mostly at $\sim 135^\circ$ were preferentially severed as in previous results, ACPs bearing large loads unbound from actin segments oriented mostly at $\sim 45^\circ$ that correspond to the supportive framework (Figure 4.9b). Therefore, ACP unbinding and F-actin severing independently
Figure 4.9. F-actin severing and ACP unbinding induce distinct modes of stress relaxation in a network. (a) Stress measured from networks with only severing (red circles), only ACP unbinding (blue triangles), and both (green inverted triangles) in response to cyclic shear strain (black). Higher cross-linking density was used ($R_{ACP} = 0.1$) compared to that of the previous cases (0.032). With only ACP unbinding, stress at 10 s is smaller, but it is maintained well over cycles. Stress relaxation observed in the case with only severing still emerges in the case with both severing and ACP unbinding, implying that they do not interfere with each other. (b) Orientation of actin segments from which ACPs unbind with high loads ($\| \mathbf{F}_{s,ACP} \| \geq 8 \text{ pN}$) (black) or which are severed (white) in a single network at $t < 10$ s. ACP unbinding predominantly occurs in the supportive framework ($\sim 45^\circ$), whereas severing occurs mostly outside the supportive framework ($\sim 135^\circ$). (All parts were reprinted from [14].)

facilitate distinct modes of stress relaxation in a single network. Since both ACP unbinding and F-actin severing events are modeled as stochastic events, an increase in a strain rate decreases stress relaxation induced by both events (Figures 4.5c, d). It has been shown that above certain strain levels, strain-softening occurs due to catastrophic ACP unbinding [17, 76], which may irreversibly alter structural organization of the network and thus lead to stress relaxation over cycles. However, this study employed relative small strain amplitudes and a low strain rate, which enables networks to be self-healed via subsequent binding and unbinding of ACPs. Thus, ACP unbinding induces stress relaxation during the advance of current strain-stiffening, but stress is hardly relaxed over cycles. By contrast, F-actin severing leads to minimal
stress relaxation during the current strain-stiffening but results in substantial stress relaxation when strain is reversed to an opposite sign.
5. MORPHOLOGICAL TRANSFORMATION AND FORCE GENERATION OF
ACTIVE CYTOSKELETAL NETWORKS

(Most of this section is adopted from [23])

The actin cytoskeleton plays an important role in various cellular processes, such as changes in cell shape, cytokinesis, and cell migration [2]. Much of the mechanical forces required for these processes are generated by interactions between actin filaments (F-actin) and myosin II motors [77] Actomyosin contractility regulates structural organization of the actin cytoskeleton and its rheological properties by interacting and competing with the dynamics of actin cross-linking proteins (ACPs) and actin filaments. For example, during Dictyostelium furrow ingression, interactions between myosin and ACP dynamics control cytokinesis contractility dynamics and mechanics [78]. In addition, during fission yeast cytokinetic ring assembly, an increase in ACP density prevents clump formation [79,80]. Representative cytoskeletal structures that are regulated by actomyosin contractility are various types of bundles, such as stress fibers, random polarity bundles, cytokinetic rings, and transverse arcs [81]. Despite similarity in their structural organization, these bundles are formed via very distinct mechanisms. Dorsal stress fibers are assembled via formin-driven polymerization of actin filaments occurring outside adhesion sites. Transverse arcs, that are located at the interface between lamellipodia and lamella, form via actomyosin-driven condensation of actin filaments within the lamellipodia [82]. During the condensation, actin filaments whose barbed ends are initially biased toward the cell margin are reoriented and thus become parallel to the margin. Transverse arcs move away from the cell margin and eventually coalesce with dorsal stress fibers, to transmit contractile forces to surrounding environments, without direct attachment to focal adhesions [83].

Several aspects regarding structural reorganization of a network into a bundle have been investigated in previous numerical studies. It was shown that an increase
in myosin density induces a structural transition from networks into bundles through a series of hierarchical steps [84] with enhancement of forces generated by the acto-myosin structures [53]. In addition, a recent study demonstrated that an increase in ACP density above a threshold value leads to a switch-like transition from random networks to ordered, bundled structures [85]. However, owing to the highly simplified models and limited scopes of the previous studies, it still remains inconclusive how a network is transformed into a bundle, how force is generated, and what happens on actin filaments during the structural reorganization. Several biophysical factors are likely to impact network transformation into a bundle. For example, an extent to which actin filaments are cross-linked will play an important role. If filaments are loosely cross-linked, they may be reoriented relatively easily to form a bundle, but low network connectivity could be antagonistic to the stability of formed bundles and generated forces. By contrast, if actin filaments are heavily cross-linked, they may not easily rotate without significant deformation. Because of the low bending rigidity of actin filaments, myosin motor activity could result in buckling during reorientation and compaction of cross-linked actin filaments. As suggested by a previous theoretical study [51], filament buckling may play a critical role in either force generation or bundle formation or in both. In addition, fast turnover of actin filaments occurring via diverse actin binding proteins within cells has potential to modulate the morphological transformation and force generation. Using only experiments, it is challenging to accurately evaluate relative importance of each of these factors and isolate their effects.

In this work, using the agent-based computational model introduced in the methods section, we systematically investigated morphological transformation of an acto-myosin network into a bundle and force generation during the transformation. We investigated effects of diverse biophysical parameters on network compaction into a bundle, which were not systematically studied in previous computational works. Specifically, we focused on the impacts of the densities of ACPs and motors and of the rigidity, initial orientation, and turnover of actin. Results from the study were
discussed in the context of the assembly of transverse arcs observed in migrating cells [82]. This study provides new insights into mechanistic understanding of a role of the interplay between various biophysical factors in bundle formation and force generation.

5.1 Methods

5.1.1 Model overview

Using the computational model introduced in the methods section, we formed a homogenous actomyosin network consists of F-actins, ACPs and motors (Figure 5.1) in a three-dimensional rectangular computational domain $(4 \times 8 \times 0.5 \, \mu m)$. A periodic boundary condition is imposed in the y-direction, whereas boundaries in the x- and z-directions exert repulsive forces on the model components to keep them within the domain. After network assembly, motors start walking on actin filaments, facilitating transformation of the network to a bundle. We measured a macroscopic force generated by a bundle and microscopic forces acting on ACPs and motors.

5.1.2 Actin dynamics

In the model, actin experiences nucleation, polymerization, and depolymerization. Nucleation corresponds to de novo appearance of one actin segment. Polymerization and depolymerization are implemented by adding and removing one actin segment on filaments, respectively. We simulated treadmilling of actin filaments by imposing equal polymerization and (reference) depolymerization rate at barbed and pointed ends, respectively. A turnover rate indicates how fast an actin filament turns over, which is equal to either polymerization or depolymerization rate. We chose physiologically relevant turnover rates $(30-120 \, s^{-1})$. A nucleation rate is also adjusted to maintain a relatively constant actin filament length. We assumed that actin nucleation takes place in the y-direction within a bundle.
Figure 5.1. An example of bundle formation via compaction of a homogeneous network. The network \((4 \times 8 \times 0.5 \, \mu m)\) consists of actin (teal) with concentration \(C_A = 40 \, \mu M\), motor (red) with density \(R_M = 0.08\), and ACP (yellow) with density \(R_{ACP} = 0.02\). A periodic boundary condition is applied only in the y-direction. After motors start walking at \(t = 0\) s, initially homogeneous network compacts into a bundle within 20 s. (Reprinted from [23].)

It is assumed that depolymerization can be inhibited by bound ACPs or motors [86]; an inhibition factor ranging between 0 and 1 \((\zeta_{d,A})\) determines the extent of inhibition:

\[
k_{d,A} = k_{d,A}^0 (1 - \zeta_{d,A})
\]

where \(k_{d,A}^0\) and \(k_{d,A}\) are reference and adjusted depolymerization rates at a barbed end or a pointed end. Thus, \(\zeta_{d,A} = 0\) corresponds to no depolymerization inhibition, whereas \(\zeta_{d,A} = 1\) means complete inhibition.

5.1.3 Network preassembly

We used a 3D rectangular computational domain \((4 \times 8 \times 0.5 \, \mu m)\) with a periodic boundary condition in the y-direction. Self-assembly of actin filaments, ACPs, and
motors in the domain results in a homogenous actomyosin network. During the network assembly, actin monomers are nucleated and polymerized into filaments. When creating anisotropic networks, direction of nucleation is controlled so that actin filaments lie along desired directions after network assembly. Motors are assembled into thick filaments, and motor arms bind to actin filaments without walking motion. ACPs bind to actin filaments to form functional cross-links between pairs of actin filaments. Due to the fixed ratio of nucleation rate to turnover rate, the average length of actin filaments is maintained at \( \sim 1.56 \mu \text{m} \). After the network assembly, motors start walking on actin filaments, and the nucleation rate is dynamically controlled to maintain the average filament length at a constant level. Actin monomer concentration \((C_A)\) is 40 \(\mu\)M for all cases.

5.1.4 Measurement of force and sustainability

To measure tension generated by a bundle, we consider 10 cross-sections that are regularly located in the computational domain in the y-direction. Tension is calculated by summing the normal component of extensional forces of all constituents crossing a cross-section. We repeat this calculation on 10 cross-sections and compute the average. Sustainability of the tension is calculated in the same manner as in [87].

5.1.5 Quantification of formation time and shape of bundles

We measured time evolution of standard deviation of x positions of actins \((\sigma_x)\). \(\sigma_x\) decreases as a bundle forms and then either remains relatively constant until the end of the simulations or increases slowly over time if the bundle is disintegrated. As a measure of how fast a network compacts into a bundle, we define the compaction time as time when the rate of change in x becomes larger than 0.01 \(\times\) (the average rate of change in \(\sigma_x\) during first 5s). In addition, we used the magnitude of the standard deviation at the same time point \((\sigma_x^c)\) as a measure of how tightly the bundle is formed in the x-direction.
5.2 Results

5.2.1 Densities of motors and ACPs critically regulate bundle formation and tension generation

Consistent with previous theoretical and experimental studies \[24,39,88\], densities of ACPs \((R_{ACP})\) and motors \((R_M)\) critically affect bundle formation and tension generation. With \(R_M = 0.08\) and \(R_{ACP} = 0.01\), a homogeneous network compacted into a bundle spanning the computational domain in the y-direction within \(\sim 10\) s (Figure 5.2a). However, the bundle was heterogeneous at 10 s in terms of actin concentration, showing a few regions with higher actin density. In addition, the bundle was highly unstable, resulting in a few separate aggregates over time. Tension measured in the bundle increased up to \(\sim 0.8\) nN and then decreased to nearly zero (Figure 5.2c). By contrast, with \(R_M = 0.08\) and \(R_{ACP} = 0.1\), a more compact, uniform bundle was formed within 15 s, and the bundle remained intact for the duration of the simulation (Figure 5.2b). Tension increased up to \(\sim 4\) nN, and then decreased slowly. Microscopic forces exerted on each motor \((f_{M}^{\text{max}})\) and ACP \((f_{ACP}^{\text{max}})\) measured at maximum tension can explain the magnitude and sustainability of the generated tension (Figure 5.2d). Note that and are positive when they are exerted toward barbed ends of actin filaments. With a large number of ACPs \((R_M = 0.08\) and \(R_{ACP} = 0.1\)), was higher, and was smaller. If there are many ACPs, they share loads exerted by motors, leading to smaller force on each ACP. Since ACPs are assumed to exhibit slip-bond behavior, the smaller force on ACPs leads to less frequent unbinding events of ACPs. Thus, stable ACPs can help motors to generate higher force close to their stall force and support the force for a longer time. By contrast, with fewer ACPs \((R_M = 0.08\) and \(R_{ACP} = 0.01\)), most motors failed to attain their stall force, and each ACP supported a larger force, leading to instability of the bundle and reduction in generated tension (Figure 5.2d).

We systematically varied \(R_{ACP}\) and \(R_M\) to probe their effects on bundle formation and tension generation. Maximum tension was positively correlated with both
Figure 5.2. Densities of motors ($R_M$) and ACPs ($R_{ACP}$) determine characteristics of bundle formation and tension generation. (a-b) Snapshots showing actin density for (a) unsuccessful ($R_M = 0.08, R_{ACP} = 0.01$) and (b) successful bundle formation ($R_M = 0.08, R_{ACP} = 0.1$). (c) Time evolution of tensile forces generated by bundles shown in (a) and (b). (d) Distribution of forces exerted on motors ($f^\text{max}_M$) and ACPs ($f^\text{max}_{ACP}$) measured at peak tension for cases shown in (a) and (b). The gray dashed line indicates stall force of motors (5.7pN). (e) The maximum and (f) sustainability of tensile forces generated by bundles, depending on $R_M$ and $R_{ACP}$. The sustainability ranges from 0 (not sustainable at all) to 1 (perfectly sustainable). (f) Compaction time as a measure of how rapidly networks transform into bundles. (g) Standard deviation of x positions of actins at compaction time ($\sigma^x_c$) as a measure of how tightly the bundle is formed. (All parts were reprinted from [23].)
densities (Figure 5.2e), whereas sustainability was proportional to $R_{ACP}$ but inversely proportional to $R_M$ (Figure 5.2f). We measured time evolution of standard deviation of x positions of actins ($\sigma_x$) to quantify compaction of networks (Figure 5.3). $\sigma_x$ tends to initially decrease, indicating compaction of networks. After reaching its minimum value, $\sigma_x$ remained constant in most cases. However, in some cases, $\sigma_x$ increased over time, which may indicate disintegration of a bundle into aggregates. Indeed, the increase in $\sigma_x$ occurred in cases with higher $R_M$ and lower $R_{ACP}$ where tension is not sustained well, and bundles are likely to form aggregates. In cases with very low $R_M$, $\sigma_x$ continuously decreased, indicating very slow compaction of networks. To quantify how fast networks compact, we defined compaction time as time at which the rate of change in $\sigma_x$ over time becomes larger than 0.01 (the average rate of change in $\sigma_x$ during first 5s). The compaction time was shorter at higher $R_M$ and lower $R_{ACP}$ (Figure 5.2g). We used the standard deviation at compaction time ($\sigma_x^c$) as an indicator of how tightly a network is compacted in the x-direction (Figure 5.2h). A tighter bundle was formed with higher $R_M$ and $R_{ACP}$. A sufficient amount of ACPs can tighten bundles by helping force generation of motors and increasing connectivity of bundles. However, ACPs slow down formation of bundles because a network becomes much more stiffer with more ACPs. In sum, a network with more motors compacted faster into a tighter bundle exerting larger tension because there are more force generators. However, the bundle and the tension are likely to be unstable, leading to bundle disintegration into aggregates and significant tension relaxation. A network with more ACPs compacted more slowly into a tighter bundle generating larger and more sustained tension.

5.2.2 Buckling of actin filaments is crucial for the transformation into a bundle

In our previous studies, it was shown that buckling of actin filaments is necessary for contraction of a network and for force generation in a preformed bundle [13, 24].
Figure 5.3. Time evolution of standard deviation of x positions of actins ($\sigma_x$) for the cases shown in Figure 5.2. ACP density used in these cases is (a) 0.01, (b) 0.018, (c) 0.032, (d) 0.056, and (e) 0.1. Motor density is 0.0008 (red), 0.0026 (blue), 0.008 (green), 0.026 (cyan), and 0.08 (black). (All parts were reprinted from [23].)
We quantified buckling events occurring in the simulations shown in Figure 5.2e-g, by tracking the ratio of end-to-end distance to contour length of actin filaments. Since most actin filaments have multiple, transiently bound motors and ACPs, buckling takes place in various ways; some of the actin filaments experienced subsequent buckling events at multiple locations over time, and buckled filaments, at times, became straight again (Figure 5.4). We determined the number of actin filaments that undergo buckling at least once in each simulation by assuming that actin filaments with a ratio of end-to-end distance to contour length smaller than 0.6 are buckled. We found that buckling occurred less frequently with higher \( R_{ACP} \) because the critical force above which buckling occurs becomes larger with higher \( R_{ACP} \) (Figure 5.5a); this is associated with a decrease in distance between adjacent cross-linking points on an actin filament. Although motors generate larger forces with higher \( R_{ACP} \) (Figure 5.2d), the increase in the critical force required for buckling is greater, leading to less frequent buckling events. With higher \( R_M \), buckling took place more frequently since more motors generate larger contractile forces that can induce buckling. These buckling events mostly occurred during the transformation to a bundle before tension reached its maximum, rather than after the peak tension (Figure 5.5d).

We tested whether buckling is required for the transformation of a network into a bundle by suppressing the filament buckling via a 100-fold increase in the bending stiffness of actin filaments \( (\kappa_{b, A} = 100 \times \kappa_{b, A}^*) \), where \( \kappa_{b, A}^* \) is reference bending stiffness. At both high and low levels of \( R_{ACP} \), a bundle rarely formed although some of the actin filaments formed a pseudo bundle at the center (Figures 5.5b, c). At \( R_M = 0.08 \) and \( R_{ACP} = 0.1 \), the developed tension in a network with \( 100 \times \kappa_{b, A}^* \) was much smaller than that with \( \kappa_{b, A}^* \), and buckling rarely occurred (Figure 5.5d). Smaller tension for the case with \( 100 \times \kappa_{b, A}^* \) can be attributed to low values of \( f_{M}^{\text{max}} \); although some values reached stall force, there was a general tendency for the forces to be smaller overall than those in the case with \( \kappa_{b, A}^* \) (Figure 5.5e). Negative values of \( f_{ACP}^{\text{max}} \) were also slightly smaller in magnitude for the case with \( 100 \times \kappa_{b, A}^* \) since ACPs sustain lower positive \( f_{M}^{\text{max}} \) in this case. Note that negative or positive \( f_{ACP}^{\text{max}} \).
Figure 5.4. Buckling of actin filaments in the case shown in Figure 5.2b. (a) The ratio of end-to-end distance to contour length of two selected actin filaments. The actin filament represented by red experienced a sequence of buckling events at around $t = 8$, 25, 50, and 80 s, whereas the actin filament represented by blue underwent buckling at around $t = 8$ s and was straightened at around $t = 75$ s. (b, c) Visualization of (b) subsequent buckling events and (c) straightening of buckled actin filaments shown in (a). Solid circles located at the ends of the actin filaments represent their barbed ends. (All parts were reprinted from [23].)
Figure 5.5. Buckling of actin filaments plays a crucial role in bundle formation and tension generation. (a) Number of actin filaments that experience buckling at least once during simulation depending on densities of motors ($R_M$) and ACPs ($R_{ACP}$), normalized by the largest number. (b-c) Snapshots showing actin density of networks where buckling is suppressed via a 100-fold increase in bending stiffness of actin filaments ($\kappa_{b,A}$). (d) Time evolution of generated tension (solid line) and the number of buckling events (dashed line) for cases with reference bending stiffness (blue triangle, $\kappa_{b,A} = \kappa_{b,A}^*$) and 100-fold higher bending stiffness (red circle, $\kappa_{b,A} = 100 \times \kappa_{b,A}^*$) at $R_M = 0.08$ and $R_{ACP} = 0.1$. (e) Distribution of forces exerted on motors ($f_{\text{max}}^M$) and ACPs ($f_{\text{max}}^{ACP}$) measured at peak tension for cases shown in (d). Legend is shared with (d). (f) The maximum and (g) sustainability of tension measured from cases ($\kappa_{b,A} = 100 \times \kappa_{b,A}^*$) with various $R_M$ and $R_{ACP}$. (h) Compaction time. (i) Standard deviation of x positions of actins at compaction time ($\sigma_{x}^c$). (All parts were reprinted from [23].)
sustain positive or negative $f_{M}^{\text{max}}$, respectively. Positive $f_{M}^{\text{max}}$ showed higher value for the case with $100 \times \kappa_{b,A}^{*}$, since this case exhibits a significant amount of negative $f_{M}^{\text{max}}$ while the case with $\kappa_{b,A}^{*}$ does not.

Due to the catch-bond nature of motors, the lower positive $f_{M}^{\text{max}}$ makes motors stay for a shorter time on actin filaments, which corresponds to a lower duty ratio of motors. Then, motors are less able to stably generate a large amount of forces. Suppression of bundle formation and generation of lower tension observed in Figures 5.5b-d might originate largely from a decrease in the duty ratio rather than an increase in $\kappa_{b,A}$. To confirm the importance of $\kappa_{b,A}$, we ran a simulation using motors with a much higher unbinding rate (i.e. lower duty ratio) than the case shown in Figure 5.2b where a stable bundle was formed. We varied one of the mechanochemical rates in the parallel cluster model [28,29], which leads to a decrease in the stall force from 5.7 pN to 5.3 pN and an increase in the unbinding rate from 0.049 s$^{-1}$ to 0.49 s$^{-1}$. As shown in Figure 5.6, a bundle still formed well, and tension inside the bundle and sustainability was similar to that of the reference case shown in Figures 5.2b, c. Thus, the inhibition of bundle formation and the decrease in tension result mostly from the change in the $\kappa_{b,A}$, not the change in the duty ratio of motors.

Maximum tension measured under various values of $R_{M}$ and $R_{ACP}$ with $100 \times \kappa_{b,A}^{*}$ (Figure 5.5f) was much lower than that measured with $\kappa_{b,A}^{*}$ (Figure 5.5e). Dependences of sustainability and compaction time on $R_{M}$ and $R_{ACP}$ (Figures 5.5g, h) were similar to those in the cases with $\kappa_{b,A}^{*}$ (Figures 5.2f, g). We also measured time evolution of $\sigma_{x}$ for quantification of network compaction (Figure 5.7). Interestingly, in cases with lower $R_{ACP}$ and high $R_{M}$, $\sigma_{x}$ increases beyond its initial value after reaching the minimum. $\sigma_{x}^{c}$ was overall higher in the cases with $100 \times \kappa_{b,A}^{*}$ (Figure 5.5i) than that in the cases with $\kappa_{b,A}^{*}$ (Figure 5.2h), quantitatively showing suppression of bundle formation with stiffer actin filaments. Interestingly, with more ACPs, $\sigma_{x}^{c}$ was larger, which is opposite to the observation in Figure 5.2h. As shown in Figure 5.5a, buckling occurred less frequently at higher $R_{ACP}$ even with $\kappa_{b,A}^{*}$. However, since a fraction of actin filaments were still buckled, the number of buckled actin...
Figure 5.6. Effects of duty ratio of motors on bundle formation and tension generation. Densities of motors and ACPs are 0.08 and 0.1, respectively. Compared to the reference case with same $R_M$ and $R_{ACP}$ (Figure 5.2b), stall force of motors was decreased from 5.7 pN to 5.3 pN, and unbinding rate was increased from 0.049 s$^{-1}$ to 0.49 s$^{-1}$. (a) Snapshots showing actin density in the networks at $t = 10$, 30, and 60 s. A bundle forms well as in the reference case. Time evolution of (b) tension and (c) standard deviation of x positions of actins ($\sigma_x$) which shows similar tendency to that in the reference case. (All parts were reprinted from [23].)
filaments is not a critical factor determining the extent of network compaction. By contrast, with $100 \times \kappa_{b,A}^*$, most of actin filaments cannot be buckled due to a significant increase in the critical buckling force. Then, network compaction becomes very sensitive to the number of buckled actin filaments because buckling is necessary for network compaction, resulting in less network compaction with higher $R_{ACP}$.

![Figure 5.7](image)

Figure 5.7. Time evolution of standard deviation of x positions of actins ($\sigma_x$) for the cases shown in Figure 5.5. Density of ACPs used in these cases is (a) 0.01, (b) 0.032, and (c) 0.1. Motor density is 0.0008 (red), 0.0026 (blue), 0.008 (green), 0.026 (cyan), and 0.08 (black). (All parts were reprinted from [23].)

In sum, these results demonstrate that even with a sufficient number of ACPs that sustain tension and help motors reach their stall force, buckling of actin filaments is required for formation of tight bundles and generation of large tension.

5.2.3 Initial orientation of actin filaments regulates bundle formation and tension generation

Myosin II motors compact actin filaments in lamellipodia into transverse arcs that generate contractile forces [89]. Since the barbed ends of all actin filaments in lamellipodia are directed toward the cell margin, the lamellipodia is not an isotropic actin network. We probed the effects of anisotropic initial orientations of actin filaments on bundle formation and tension generation with $R_M = 0.08$ and $R_{ACP} = 0.01$ by creating three networks consisting of actin filaments with biased initial orientations
(Figures 5.8a-c). Note that the case shown in Figure 5.8b where actin filaments are initially oriented toward the +x direction mimics filament orientation in lamellipodia. Compared to the reference case with isotropic orientation of filaments (Figures 5.8a, c), the networks with biased orientations showed lower maximum tension and slower bundle formation (Figures 5.8a-d) because there were a smaller number of antiparallel pairs of actin filaments that are in configuration suitable for motors to produce force (Figures 5.8f). Interestingly, a network with barbed ends directed toward +y was effectively transformed to a bundle with significant tension despite the fact that it initially had no antiparallel pairs of actin filaments in the y-direction. We found that some of the actin filaments changed their orientations (Figure 5.9 and Figure 5.8c, right column) during network contraction (Figure 5.8f). Even in the network with barbed ends oriented toward +x/+y, a bundle could form slowly and generate tension due to changes in filament orientation (Figures 5.8a, d, f). In all cases, bundles eventually collapsed into a few aggregates; this occurred at a rate proportional to the maximum tension because larger tension accelerates destabilization of ACPs, leading to faster disintegration of bundles. We also tested the influence of initial orientation of actin filaments (diagonal or horizontal/vertical) on bundle formation and tension generated in networks, and the results overall showed similar tendencies (Figures 5.10, 5.11). At higher ACP density ($R_M = 0.08$ and $R_{ACP} = 0.1$), actin filaments tend to rotate less than those at lower $R_{ACP}$ because the filaments are confined more by a larger number of ACPs (Figures 5.12a, b, c). However, some of the actin filaments were still able to change their orientations, contributing to tension generation (Figures 5.12d, e). Note that unlike the case with lower ACP density, the bundles were not disintegrated into aggregates, regardless of initial filament orientation. This can explain a discrepancy between the unstable bundle shown in Figure 5.8b formed from networks mimicking geometry of lamellipodia and a stable bundle observed at the interface between lamellipodia and lamella. It is expected that actin filaments with numerous branching points in lamellipodia have very high connectivity between actin filaments, preventing a bundle from being disintegrated.
Figure 5.8. Densities of motors ($R_M$) and ACPs ($R_{ACP}$) used in cases shown here are 0.08 and 0.01, respectively. (a-c) (1st column) Orientations where barbed ends of actin filaments in networks are initially directed. Red on the circles located at the bottom-right corner represents the range of the orientation. Arrows in the boxes represent examples of filaments with corresponding initial orientations. (2nd, 3rd, 4th columns) Snapshots showing actin density in the networks at $t = 10$, 30, and 60 s with initial orientation indicated in the 1st column. (5th column) Initial and final orientations of actin filaments. Final orientation indicates orientation of filaments measured at a time point when compaction time is defined. (d) Time evolution of tension for cases with biased initial orientations shown in (a-c) and isotropic initial orientation. (e) Number of buckling events occurring during simulation for cases shown in (d). (f) Time evolution of a fraction of antiparallel filament pairs for cases shown in (d). (All parts were reprinted from [23].)
Figure 5.9. Rotation of actin filaments in the case shown in Figure 5.8c. (a) Time evolution of orientation of two selected actin filaments. At around \( t = 20 \) s, both actin filaments rotate by about \( 180^\circ \) (b, c) Visualization of rotation of the actin filaments shown in (a). Solid circles located at the ends of the actin filaments represent their barbed ends. (All parts were reprinted from [23].)
Figure 5.10. Effects of initial orientation of diagonally nuclearized actin filaments on bundle formation and tension generation. Densities of motors and ACPs used in cases shown here are 0.08 and 0.01, respectively. (a-d) (1st column) Orientations where barbed ends of actin filaments in networks are initially directed. Red lines on the circles located at the bottom-right corner represent the orientations. Arrows in the boxes represent examples of filaments with corresponding initial orientations. (2nd, 3rd, 4th columns) Snapshots showing actin density in the networks at $t = 0$, 10, and 40 s with initial orientation indicated in the 1st column. (e) Time evolution of tension for cases shown in (a-d). (f) Time evolution of orientations of selected actin filaments in the case shown in (a). (g) Time evolution of a fraction of antiparallel filament pairs for cases shown in (a-d). (h) Number of buckling events occurring during simulations for cases shown in (a-d). (All parts were reprinted from [23].)
Figure 5.11. Influences of initial orientation of perpendicularly nuclearized actin filaments on bundle formation and tension generation. Densities of motors and ACPs used in cases shown here are 0.08 and 0.01, respectively. (a-d) (1st column) Orientations where barbed ends of actin filaments in networks are initially directed. (2nd, 3rd, 4th columns) Snapshots showing actin density in the networks at $t = 0, 10,$ and $40$ s with initial orientation indicated in the 1st column. (e) Time evolution of tension for cases shown in (a-d). (f) Time evolution of orientation of selected actin filaments from case shown in (a). (g) Time evolution of a fraction of antiparallel filament pairs for cases shown in (a-d). (h) Number of buckling events occurring during simulations for cases shown in (a-d). (All parts were reprinted from [23].)
Figure 5.12. Effects of initial orientation of actin filaments on bundle formation and tension generation in networks with numerous ACPs. Densities of motors and ACPs used in cases shown here are 0.08 and 0.1, respectively. (a-c) (1st column) Orientations where barbed ends of actin filaments in networks are initially directed. (2nd, 3rd, 4th columns) Snapshots showing actin density in the networks at $t = 10$, 30, and 60 s with initial orientation indicated in the 1st column. (5th column) Initial and final orientations of actin filaments. Final orientation indicates orientation of filaments measured at a time point when compaction time is defined. (d) Time evolution of tension for cases with biased initial orientations shown in (a-c) and isotropic initial orientation. (e) Time evolution of a fraction of antiparallel filament pairs for cases shown in (d). (All parts were reprinted from [23].)
Taken together, these results demonstrate that networks with biased filament orientations can still be transformed to bundles owing to changes in filament orientation occurring during contraction. However, if orientations are biased, bundles are loose, and generated tension tends to be lower but is sustained for a longer time.

5.2.4 Buckling is not necessary for bundle formation in networks with biased filament orientation

We have observed that buckling is necessary for bundle formation in networks with isotropic filament orientation since contraction of antiparallel pairs of actin filaments requires buckling. We tested whether buckling is still necessary for bundle formation in networks with a much smaller number of antiparallel pairs by increasing the bending stiffness of actin filaments 100-fold as before ($100 \times \kappa_{b,A}^*$). We found that networks with barbed ends directed toward $+x/+y$ or $+y$ were still transformed to bundles because contraction in the y-direction does not need to occur in such configurations (Figure 5.13a, c). Filaments in the network with barbed ends directed toward $+x/+y$ initially form only parallel pairs of actin filaments, so they can be aligned in the y-direction (Figure 5.14a). Filaments forming antiparallel pairs in the x-direction in the network with barbed ends directed toward $+y$ can be aligned in the y-direction via polarity sorting due to the absence of a periodic boundary condition in the x-direction (Figure 5.14c). Some of the filaments changed their orientation during bundle formation, resulting in antiparallel pairs in the y-direction that were also connected to other actin filaments in a bundle (Figure 5.13e). Due to suppression of buckling, these pairs cannot contract, so the bundles remained curved rather than straight. Accordingly, forces generated on bundles remained close to zero and even became compressive (i.e. negative) (Figure 5.13d). By contrast, a network with barbed ends directed toward $+x/\pm y$ could not form a bundle since the antiparallel pairs of filaments that existed from the beginning were not able to contract (Figure 5.13b and Figure 5.14). Tension generated in these networks was similar to that
in networks with isotropic orientations (Figure 5.13d). Therefore, buckling is not always necessary for the transformation of a network to a bundle. If orientation of actin filaments is highly anisotropic, the transformation can still take place via polarity sorting of filaments by motors. However, tensile forces are not developed on the formed bundles.

5.2.5 Actin turnover modulates bundle formation and tension generation

In our previous study, we demonstrated that actin turnover modulates the buildup and sustainability of tension generated by actomyosin networks [87]. We tested effects of actin turnover on bundle formation and tension generation by imposing actin treadmilling at various rates \( (k_{t,A}) \) under a condition where bundles generate unsustainable tension and eventually form aggregates in the absence of any turnover \( (R_M = 0.08 \) and \( R_{ACP} = 0.01 \)). We additionally assumed that depolymerization of actin filaments can be inhibited by bound ACPs or motors to a different extent [77]. We define the inhibition factor \( (\zeta_{d,A}) \) to represent this effect; with \( \zeta_{d,A} = 0 \), depolymerization is not inhibited at all, whereas inhibition is complete with \( \zeta_{d,A} = 1 \). In a control case without turnover \( (k_{t,A} = 0) \) and a case with \( k_{t,A} = 60 \text{ s}^{-1} \) and \( \zeta_{d,A} = 1 \), bundles became aggregates within 100 s (Figure 5.15a, d), and generated tension fell to nearly zero (Figure 5.15e). With \( k_{t,A} = 60 \text{ s}^{-1} \) and \( \zeta_{d,A} = 0 \), some of the actin filaments in the network formed a thin bundle that was converted into aggregates over time (Figure 5.15b), and tension ultimately relaxed to zero (Figure 5.15e). By contrast, with \( k_{t,A} = 60 \text{ s}^{-1} \) and \( \zeta_{d,A} = 0.6 \), the bundle was maintained much longer, showing highly sustainable tension (Figures 5.15c, e). We systematically probed the effects of \( k_{t,A} \) and \( \zeta_{d,A} \) on the maximum and sustainability of tension (Figures 5.15f, g). While maximum tension showed no correlation with \( k_{t,A} \) and \( \zeta_{d,A} \), sustainability tended to be higher at intermediate levels of \( \zeta_{d,A} \) because too large \( \zeta_{d,A} \) completely inhibits actin turnover, whereas too small \( \zeta_{d,A} \) precludes bundle formation and destabilizes the bundle by ACP unbinding induced by actin turnover. The region with higher
Figure 5.13. In networks with biased filament orientation, parallel filament pairs can form bundles without buckling of filaments, whereas antiparallel pairs cannot. Densities of motors ($R_M$) and ACPs ($R_{ACP}$) are 0.08 and 0.01, respectively as in Figure 5.8, but bending stiffness of actin filaments is increased 100-fold ($100 \times \kappa_{b,A}^*$). (a-c) (1st column) Initial orientations of actin filaments in networks. (2nd, 3rd, 4th columns) Snapshots showing actin density in the networks with initial filament orientation indicated in the 1st column. (5th column) Initial and final orientation of actin filaments. (d) Time evolution of tensile forces generated by bundles for cases with biased initial orientations shown in (a-c) and isotropic initial orientation. (e) Time evolution of a fraction of antiparallel filament pairs for cases shown in (d). (All parts were reprinted from [23].)
Figure 5.14. In networks with biased filament orientations, bundles can form without buckling of actin filaments. Schematic diagrams show actin filaments and motors initially directed toward (a) $+x/+y$, (b) $+x/\pm y$, and (c) $x/+y$ as in cases shown in Figures 5.8a-c. Teal and red represent actin filaments and motors, respectively, whereas ACPs are not shown for simplicity. (a) Most of the actin filaments oriented toward $+x/+y$ are aligned in parallel via polarity sorting. (b) Antiparallel pairs of actin filaments initially oriented relatively in the y-direction can be aligned well in the y-direction. However, the alignment results in the buildup of compressive forces on the actin filaments unlike in other cases. If bending stiffness of actin filaments is low enough, the actin filaments are buckled and oriented in the y-direction. If buckling is suppressed due to large bending stiffness, the actin filaments cannot be oriented in the y-direction. (c) Antiparallel pairs of actin filaments initially oriented relatively in the x-direction can be aligned in the y-direction via polarity sorting. (All parts were reprinted from [23].)
sustainability is wider with lower $k_{t,A}$, since less turnover occurs at lower $k_{t,A}$ at the same level of $\zeta_{d,A}$. Networks compacted faster with more turnover (i.e. higher $k_{t,A}$ and lower $\zeta_{d,A}$), but formed bundles were loose (Figures 5.15h, i). This agrees with the observation that compaction occurred faster, and more loose bundles formed at lower $R_{ACP}$ (Figures 5.2g, h), because more frequent turnover facilitates unbinding of ACPs, leading to a decrease in the number of active ACPs bound on two actin filaments at dynamic equilibrium. Also, with low $\zeta_{d,A}$, $\sigma_x$ increased after reaching its minimum (Figure 5.16), which corresponds to disintegration of a bundle into a network. However, the increase in $\sigma_x$ significantly slowed down after some time in several cases, which implies a steady state with coexistence of bundle and network structures as shown in Figure 5.15c.

At high $R_{ACP}$ shown in Figure 5.17 ($R_M = 0.08$ and $R_{ACP} = 0.1$), bundle formation and the maximum tension were both enhanced with slower actin turnover (i.e. lower $k_{t,A}$ and higher $\zeta_{d,A}$). Compaction time, $\sigma_x^c$, and $\sigma_x$ showed similar trends with those in Figure 5.15 (Figures 5.17, 5.18). In this case, the bundle and generated tension are already stable without turnover owing to numerous ACPs. Actin turnover decreases the number of actin filaments involved with bundle formation as can be seen in a change in the diameter of bundles (Figures 5.17b, c, d). Thus, the connectivity of filaments in the bundle is deteriorated, resulting in less sustainable tension. In addition, since turnover induces unbinding of ACPs which leads to instability, more motors failed to reach their stall force, leading to smaller maximum tension (Figure 5.17e). Indeed, $f_{MAX}^M$ was lower with increasing turnover (Figure 5.17f). $f_{MAX}^{ACP}$ also decreased with increasing turnover, owing to lower tension and facilitated ACP unbinding by actin turnover. Note that the case with $\zeta_{d,A} = 1$ showed more sustained tension than the case without actin turnover. With $\zeta_{d,A} = 1$, depolymerization occurs in regions of an actin filament which are not bound to ACPs or motors, thus unnecessary for tension generation. Depolymerized actin can be polymerized at barbed ends of actin filaments, helping sustain tension by increasing a walking distance of motors toward a barbed end. In sum, with an insufficient number of ACPs, actin turnover with
Figure 5.15. Densities of motors ($R_M$) and ACPs ($R_{ACP}$) used in cases shown here are 0.08 and 0.01, respectively. (a-d) Snapshots showing actin density in networks (a) without actin turnover and (b-d) with actin turnover rate ($k_{t,A}$) of 60 s$^{-1}$. In networks with actin turnover, depolymerization of actin filaments was inhibited by bound ACPs or motors to an extent determined by inhibition factor ($\zeta_{d,A}$). $\zeta_{d,A}$ ranges between 0 (no inhibition of depolymerization) and 1 (complete inhibition). In these examples, $\zeta_{d,A}$ is (b) 0, (c) 0.6, or (d) 1. (e) Time evolution of tensile forces generated by bundles for cases shown in (a-d). (f) The maximum and (g) sustainability of tension, depending on $k_{t,A}$ and $\zeta_{d,A}$. Maximum tension shows no correlation with $k_{t,A}$ and $\zeta_{d,A}$, whereas sustainability is higher at intermediate range of $\zeta_{d,A}$. (h) Compaction time. (i) Standard deviation of x positions of actins at compaction time ($\sigma_x$). With more turnover (i.e. high $k_{t,A}$ and low $\zeta_{d,A}$), bundles form faster, but the formed bundles are more loose. (All parts were reprinted from [23].)
Figure 5.16. Time evolution of standard deviation of x positions of actins ($\sigma_x$) in the cases shown in Figure 5.15. The turnover rate used in these cases is (a) 30 $s^{-1}$, (b) 45 $s^{-1}$, (c) 60 $s^{-1}$, (d) 75 $s^{-1}$, and (e) 90 $s^{-1}$. The inhibition factor is 0 (red), 0.2 (blue), 0.4 (green), 0.6 (cyan), 0.8 (black), and 1 (magenta). (All parts were reprinted from [23].)
intermediate values of $\zeta_{d,A}$ enhances the stability of bundles and generated tension, whereas with more ACPs, actin turnover plays only a negative role for the stability of bundles and tension.

Figure 5.17. Impacts of actin turnover on bundle formation and tension generation in networks with numerous ACPs. Densities of motors and ACPs used in cases shown here are 0.08 and 0.1, respectively. (a-d) Snapshots showing actin density in networks (a) without actin turnover and (b-d) with actin turnover rate ($k_{t,A}$) of 60 s$^{-1}$. In networks with actin turnover, depolymerization of actin filaments was inhibited by bound ACPs or motors to an extent determined by inhibition factor ($\zeta_{d,A}$). $\zeta_{d,A}$ ranges between 0 (no inhibition of depolymerization) and 1 (complete inhibition). In these examples, $\zeta_{d,A}$ is (b) 0, (c) 0.5, or (d) 1. (e) Time evolution of tensile forces generated by bundles for cases shown in (a-d). (f) Distribution of forces exerted on motors ($f_{M}^{\text{max}}$) and ACPs ($f_{ACP}^{\text{max}}$) measured at peak tension for cases shown in (a-d). The gray dashed line indicates stall force of motors (5.7pN). The legend is shared with (e). (g) The maximum and (h) sustainability of tension, depending on $k_{t,A}$ and $\zeta_{d,A}$. (i) Compaction time. (j) Standard deviation of x positions of actins at the compaction time ($\sigma_{x}^{c}$). (All parts were reprinted from [23].)
Figure 5.18. Time evolution of standard deviation of x positions of actins ($\sigma_x$) in the cases shown in Figure 5.17. The turnover rate used in these cases is (a) 60 s$^{-1}$, (b) 90 s$^{-1}$, and (c) 120 s$^{-1}$. The inhibition factor is 0 (red), 0.25 (blue), 0.5 (green), 0.75 (cyan), and 1 (black). (All parts were reprinted from [23].)

5.3 Discussions

Structural reorganization of a cross-linked actin network into a bundle occurs in several cellular phenomena, such as formation of transverse arcs at the interface between lamellipodia and lamella. Recent experiments have shown that in the absence of stress fibers, cells can still exert large tensions on surrounding environments due to contractile lamella that contain transverse arcs, implying the significance of transverse arcs in cells as a force generator [90]. To illuminate mechanisms of formation and force generation of transverse arcs, we here presented a computational study regarding transformation of actomyosin networks into bundles under diverse conditions. Results from this study demonstrate that formation of contractile bundles and force generation in the bundles are tightly regulated by the interplay between concentrations of cytoskeletal elements and the deformability, dynamics, and initial orientation of actin filaments that have not been tested systematically in previous studies. This study is significantly different from our previous study that employed actomyosin bundles preassembled by stacking straight actin filaments in parallel [24] since actin filaments are not stacked merely without any deformation during the morphological
transformation. We found that during the transition from a network into a bundle, actin filaments undergo buckling and reorientation in various ways, and a large portion of tension is built during the structural reorganization rather than after bundle formation. In addition, we incorporated systematic variations of initial filament orientation that have not been included in our previous studies [15, 17, 24, 87, 91, 92], motivated by observation that transverse arcs located at the interface between lamellipodia and lamella are formed by compaction and realignment of actin filaments with biased orientations within the lamellipodia [93].

We investigated how the density of ACPs and motors and the buckling of actin filaments govern the bundle formation and tension generation. It was found that maximum bundle tension is proportional to motor and ACP densities, whereas sustainability of tension is proportional to ACP density but inversely proportional to motor density. A key factor for determining tension sustainability is how much force is exerted on each ACP because large force can make ACPs unstable by increasing their force-dependent unbinding rate. This is consistent with our previous studies where forces are generated by cortex-like actomyosin networks [13] and preformed bundles [24].

We observed that time required for bundle formation is inversely proportional to motor density but proportional to ACP density. Previous experimental studies showed that condensation of networks into transverse arcs occurs within 20 s [94], which is comparable with the compaction time measured in this study. We also observed that buckling of actin filaments plays an important role in bundle formation, and most of the tension is generated during a transition from a network to a bundle. This is different from our previous study where we found the importance of filament buckling and force generation during contraction of the preformed bundles [24]. In addition, using networks consisting of filaments with biased orientations, we found that buckling should take place in antiparallel pairs of actin filaments initially aligned in the y-direction in order to induce transformation of networks into bundles. If there is not such an antiparallel pair in the y-direction, the transformation is possible with-
out filament buckling. However, development of large tension on a formed bundle is possible only when filament buckling is allowed. In addition, we showed that networks with isotropic filament orientations result in the best bundle formation and the largest tension. Interestingly, even if orientations of actin filaments are too biased to initially have antiparallel pairs of actin filaments, some of the actin filaments change their orientations during network contraction, resulting in antiparallel pairs and formation of bundles. However, compared to the network with isotropic orientations of actin filaments, bundles are loosely formed, and tension is smaller. Since the smaller tension leads to lower force on each ACP, tension is sustained for a longer time.

Also, we probed influences of actin turnover via treadmilling on bundle formation and tension generation as in our previous study. However, we made a new assumption that actin depolymerization rate can be varied by cross-linking points based on previous experimental observations [86]. We observed that actin turnover with moderate inhibition of actin depolymerization by motors and ACPs increases the sustainability of tension and confers structural stability to the bundles at low ACP density. If there is a selective inhibition of depolymerization, the region of a filament that contributes least to the connectivity of bundles (from a pointed end to the first cross-linking point) is depolymerized faster. Depolymerized actin can be polymerized at a barbed end of the same filament or other actin filaments. Since motors walk toward barbed ends, the newly polymerized actin can enable motors to walk further. By contrast, at high ACP density, actin turnover decreases tension sustainability and the stability of formed bundles because the connectivity of the bundles is already maximized by numerous ACPs. Loss of connectivity caused by actin turnover seems more critical than gain of stability from the turnover.

Results from this study support observations from previous studies regarding actomyosin bundles and rings. A recent study showed the importance of architecture and connectivity for the contractility of actomyosin rings [88]. This study showed that each of polarity sorting, sarcomeric contractility, and filament buckling plays an important role at low, intermediate, and high connectivity, respectively. Signifi-
cant ring contraction was observed only at regimes where sarcomeric contractility or filament buckling becomes important. Too high connectivity or too rigid filaments caused inhibition of filament buckling and ring contraction. Although we did not explore effects of very low connectivity in this study ($R_{ACP} \geq 0.01$), we observed that buckling takes place less frequently at higher ACP density (Figure 5.5a), and that suppression of buckling via an increase in filament bending stiffness results in inhibition of contraction (Figures 5.5b, c). All of these are consistent with [88] and other studies showing significance of filament buckling for contraction [95, 96]. Our study also predicted that compaction of an actomyosin network into a bundle is more significant with higher ACP and motor densities. This is in agreement with a recent computational study showing that an actomyosin network exhibits greater contraction and filament alignment with higher densities of motors and ACPs [85]. In addition, another recent computational study found that contraction of random actomyosin arrays mimicking a cytokinetic ring is slower with more cross-linkers [96], which is also consistent with our study (Figure 5.2g).
6. CONCLUSIONS

This thesis investigated mechanisms of force generation, relaxation, and remodeling of cytoskeletal networks using an agent-based computational model. In Chapter 3, the effects of motor activity and ACP dynamics on stress generation, morphology, and viscoelastic properties of cortex-like networks were investigated. It was found that ACPs transmit forces generated by motors, thus improving stability of stress generation. In contrast, it was shown that ACP unbinding disturbs stress accumulation by dissipating energy built by motors. Due to the force-dependent unbinding of ACPs, larger motor density or smaller ACP density facilitated unbinding of ACPs by increasing force acting on each ACP, thus destabilizing the stress and morphology of networks. In Chapter 4, the mechanism of stress relaxation of cross-linked actin networks subjected to cyclic shear strain was investigated. It was shown that F-actins go through buckling and thus severing during the strain-stiffening process, therefore relaxing stress exerted by networks. Furthermore, severing was found to coordinate with ACP unbinding through their differential effects on different parts of networks. Unbinding of ACPs from F-actins mostly occurred in the supportive framework, thus relaxing stress during the strain-stiffening process. F-actin severing took place outside the supportive framework, thus relaxing stress over cycles. The stress relaxation by severing was most pronounced in highly cross-linked networks with long F-actins. In Chapter 5, effects of various biophysical factors on the formation of bundles from cytoskeletal networks were investigated. It was shown that motors compact bundles fast, though the formed bundles are likely to be unstable. On the other hand, ACPs were found to compact stable bundles slowly. It was also demonstrated that buckling and turnover of F-actins are crucial for stable formation of bundles. However, bundles formed regardless of initial orientation of F-actins, owing to the reorientation of F-actins during the compaction process.
For further study, effects of molecular motors on severing of F-actins can be investigated. It has been shown that in non-muscle cells, motors generate compressive and tensile forces on F-actins [11,46]. Due to the semiflexible nature of F-actins, they can resist tension, but easily buckle under compression [11,46]. A previous study showed that myosin-induced buckling can lead to severing of F-actins [46]. It will be interesting to relate the motor induced severing of F-actins to turnover of actin bundles in migrating cells. A previous study on the neuronal growth cone suggested that myosin II activity facilitates severing and turnover of filopodia in the transition zone of cells [97]. The study demonstrated that actin retrograde flow, which is essential for cell motility, is maintained by myosin activity and actin treadmilling [97]. From the study findings, it can be inferred that the motor induced severing in transition zones possibly provides free actins to lamellipodia, thus maintaining a steady-state in a leading edge of migrating cells; however, detailed mechanisms remain unclear. In the future, contributions of motor-induced severing on regulation of actin dynamics in migrating cells will be investigated.
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