Filament turnover is essential for continuous long range contractile flow in a model actomyosin cortex.

William M McFadden[1], Patrick M McCall[2], Margaret Gardel[2], Edwin M Munro[3,*]

1 Biophysical Sciences Program, University of Chicago, Chicago, IL, USA

2 Department of Physics, University of Chicago, Chicago, IL, USA

3 Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL, USA

* emunro@uchicago.edu

Abstract

Actomyosin-based cortical flow is a fundamental engine for cellular morphogenesis. Cortical flows are generated by cross-linked networks of actin filaments and myosin motors, in which active stress produced by motor activity is opposed by passive resistance to network deformation. Continuous flow requires local remodeling through crosslink unbinding and and/or filament disassembly. But how local remodeling tunes stress production and dissipation, and how this in turn shapes long range flow, remains poorly understood. Here, we introduce a computational model for a cross-linked networks with active motors based on minimal requirements for production and dissipation of contractile stress, namely asymmetric filament compliance, spatial heterogeneity of motor activity, reversible cross-links and filament turnover. We characterize how the production and dissipation of network stress depend, individually, on cross-link dynamics and filament turnover, and how these dependencies combine to determine overall rates of cortical flow. Our analysis predicts that filament turnover is required to maintain active stress against external resistance and steady state flow in response to external stress. Steady state stress increases with filament lifetime up to a characteristic time τ_m, then decreases with lifetime above τ_m. Effective viscosity

increases with filament lifetime up to a characteristic time τ_c, and then becomes independent of filament lifetime and sharply dependent on crosslink dynamics. These individual dependencies of active stress and effective viscosity define multiple regimes of steady state flow. In particular our model predicts the existence of a regime, when filament lifetimes are shorter than both τ_c and τ_m, in which dependencies of effective viscosity and steady state stress cancel one another, such that flow speed is insensitive to filament turnover, and shows simple dependence on motor activity and crosslink dynamics. These results provide a framework for understanding how animal cells tune cortical flow through local control of network remodeling.

Author Summary

In this paper, we develop and analyze a minimal model for a 2D network of cross-linked actin filaments and myosin motors, representing the cortical cytoskeleton of eukaryotic cells. We implement coarse-grained representations of force production by myosin motors and stress dissipation through an effective cross-link friction and filament turnover. We use this model to characterize how the sustained production of active stress, and the steady dissipation of elastic stress, depend individually on motor activity, effective cross-link friction and filament turnover. Then we combine these results to gain insights into how microscopic network parameters control steady state flow produced by asymmetric distributions of motor activity. Our results provide a framework for understanding how local modulation of microscopic interactions within contractile networks control macroscopic quantities like active stress and effective viscosity to control cortical deformation and flow at cellular scales.

Introduction

Cortical flow is a fundamental and ubiquitous form of cellular deformation that underlies cell polarization, cell division, cell crawling and multicellular tissue morphogenesis [1–6]. These flows originate within a thin layer of cross-linked actin filaments and myosin motors, called the actomyosin cortex, that lies just beneath the plasma membrane [7]. Local forces produced by bipolar myosin filaments are integrated

within cross-linked networks to build macroscopic contractile stress [8–10]. At the same time, cross-linked networks resist deformation and this resistance must be dissipated by network remodeling to allow macroscopic deformation and flow. How force production and dissipation depend on motor activity and network remodeling remains poorly understood.

One successful approach to modeling cortical flow has relied on coarse-grained phenomenological descriptions of actomyosin networks as active fluids, whose motions are driven by gradients of active contractile stress and opposed by an effectively viscous resistance [11]. In these models, spatial variation in active stress is typically assumed to reflect spatial variation in motor activity and force transmission [12], while viscous resistance is assumed to reflect the internal dissipation of elastic resistance due to local remodeling of filaments and/or cross-links [7, 13]. Models combining an active fluid description with simple kinetics for network assembly and disassembly, can successfully reproduce the spatiotemporal dynamics of cortical flow observed during polarization [11], cell division [14, 15], cell motility [16, 17] and tissue morphogenesis [18]. However, it remains a challenge to connect this coarse-grained description of cortical flow to the microscopic origins of force generation and dissipation within cross-linked actomyosin networks.

Studies in living cells reveal fluid-like stress relaxation on timescales of 10-100s [1, 2, 11, 19–21], which is thought to arise through a combination of cross link unbinding and actin filament turnover [7, 13, 22]. Theoretical [23, 24] and computational [25–27] studies reveal that cross-link unbinding can endow actin networks with complex time-dependent viscoelasticity. However, while cross-link unbinding is sufficient for viscous relaxation (creep) on very long timescales *in vitro*, it is unlikely to account for the rapid cortical deformation and flow observed in living cells [26, 28–31]. Experimental studies in living cells reveal rapid turnover of cortical actin filaments on timescales comparable to stress relaxation (10-100s) [32–36]. Perturbing turnover can lead to changes in cortical mechanics and in the rates and patterns of cortical flow [34, 37]. However, the specific contributions of actin turnover to stress relaxation and how these depend on network architecture remain unclear.

Recent work has also begun to reveal mechanisms for active stress generation in
disordered actomyosin networks. Theoretical studies suggest that spatial heterogeneity
in motor activity along individual filaments, and asymmetrical filament compliance
(stiffer in extension than in compression), are sufficient for macroscopic
contraction [38, 39], although other routes to contractility may also exist [39]. Local
interactions among actin filaments and myosin motors are sufficient to drive
macroscopic contraction of disordered networks *in vitro* [40], and the kinematics of
contraction observed in these studies support a mechanism based on asymmetrical
filament compliance and filament buckling. However, in these studies, the filaments
were preassembled and network contraction was transient, because of irreversible
network collapse [41], or buildup of elastic resistance [42], or because network
rearrangements (polarity sorting) dissipate the potential to generate contractile
force [43–46]. This suggests that network turnover may play essential role(s) in allowing
sustained production of contractile force. Recent theoretical and modeling studies have
begun to explore how this might work [47–49], and to explore dynamic behaviors that
can emerge when contractile material undergoes turnover [15, 50]. However, it remains a
challenge to understand how force production and dissipation depend individually on
the local interplay of network architecture, motor activity and filament turnover, and
how these dependencies combine to mediate tunable control of long range cortical flow.

Here, we construct and analyse a simple computational model that bridges between
the microscopic description of cross-linked actomyosin networks and the coarse grained
description of an active fluid. We represent actin filaments as simple springs with
asymmetric compliance; we represent dynamic binding/unbinding of elastic cross-links
as molecular friction [51–53] at filament crossover points; we represent motor activity as
force coupling on a subset of filament cross-over points with a simple linear
force/velocity relationship [54]. Finally, we model filament turnover by allowing entire
filaments to appear with a fixed probability per unit area and disappear with fixed
probabilities per unit time. We use this model first to characterize the passive response
of a cross-linked network to externally applied stress, then the buildup and maintenance
of active stress against an external resistance, and finally the steady state flows
produced by an asymmetric distribution of active motors in which active stress and

passive resistance are dynamically balanced across the network. Our results reveal how ₆₈ network remodeling can tune cortical flow through simultaneous effects on active force ₆₉ generation and passive resistance to network deformation. ₇₀

Models

Our goal was to construct a minimal model that is sufficiently detailed to capture ₇₂ essential microscopic features of cross-linked actomyosin networks (actin filaments with ₇₃ asymmetric compliance, dynamic cross-links, active motors and and continuous filament ₇₄ turnover), but simple enough to explore, systematically, how these microscopic features ₇₅ control macroscopic deformation and flow. We focus on 2D networks because they ₇₆ capture a reasonable approximation of the quasi-2D cortical actomyosin networks that ₇₇ govern flow and deformation in many eukaryotic cells [11,55], or the quasi-2D networks ₇₈ studied recently *in vitro* [40,56]. ₇₉

Fig. 1 provides a schematic overview of our model's assumptions. We model each ₈₀ filament as an oriented elastic spring with relaxed length l_s. The state of a filament is ₈₁ defined by the positions of its endpoints $\mathbf{x_i}$ and $\mathbf{x_{i+1}}$ marking its (-) and (+) ends ₈₂ respectively. The index i enumerates over all endpoints of all filaments. We refer to the ₈₃ filament connecting endpoint i and i+1 as filament i, and we define $\mathbf{\hat{u}_i}$ to be the unit ₈₄ vector oriented along filament i from endpoint i to endpoint i+1. ₈₅

Fig 1. Schematic overview of modeling framework and assumptions. **A)** Filaments are oriented linear springs that are stiffer in extension than in compression. **B)** Cross-linking occurs at all filament crossings; we represent cross link resistance as an effective drag, proportional to the relative velocity of the overlapping filaments. **C)** We represent motor activity as a linear force-velocity relationship with a fixed force at zero velocity directed towards a filament's (-) end. We implement spatial heterogeneity by imposing motor activity at a fixed fraction of filament crossover points, resulting in variation in the magnitudes of compressive vs extensile vs translational forces along individual filament segments. **D)** Whole filaments disappear at a constant rate; new filaments appear with random positions and orientations at the constant rate per unit area, such that entire network refreshes on a characteristic timescale τ_r. **e-g)** Three different simulation scenarios: **E)** Passive response to uniaxial stress, **F)** Free contraction of an active network and **G)** Isometric contraction against a fixed boundary.

Asymmetric filament compliance

We assume (Fig. 1A) that local deformation of filament i gives rise to equal and opposite elastic restoring forces on endpoints i and i+1:

$$\mathbf{F_i^{elas}} = \mathbf{F_i^{\mu}}, \qquad \mathbf{F_{i+1}^{elas}} = -\mathbf{F_i^{\mu}} \tag{1}$$

where $\gamma_i = (|\mathbf{x_{i+1}} - \mathbf{x_i}| - l_s)/l_s$ is the strain on filament i, and the elastic modulus μ is a composite quantity that represents both filament and cross-linker compliance as in the effective medium theory of Broederz and colleagues [57]. To model asymmetric filament compliance, we set $\mu = \mu_e$ if the strain is positive (extension), and $\mu = \mu_c$ if the strain is negative (compression).

Drag-like coupling between overlapping filaments

Previous models have represented cross-linkers as elastic connections between pairs of points on neighboring filaments that bind and unbind with either fixed or force-dependent probabilities [25,57]. Here, we introduce a coarse-grained representation of crosslink dynamics by introducing an effective drag force that couples every pair of overlapping filaments, and which represents a molecular friction arising from the time-averaged contributions of many individual transient crosslinks (Fig. 1B). This is a reasonable approximation if all pairs of overlapping filaments have equal access to a non-limiting pool of cross links, and if the rate at which filaments moved past one another is slow relative to the unbinding rate of individual crosslinks [58]. This coarse-grained approach has been used to model frictional forces arising from ionic cross-linking of actin filaments *in vitro* [59,60], and simple force-velocity relationships for systems of cytoskeletal filaments and cross-linking motors [54,61–63].

To implement coupling through effective drag, for any pair of overlapping filaments i and j, we write the drag force on filament i as:

$$\mathbf{F_{i,j}^{\xi}} = -\xi(\mathbf{v_i} - \mathbf{v_j}) \tag{2}$$

where ξ is the drag coefficient and $\mathbf{v_i}$, $\mathbf{v_j}$ are the average velocities of filaments i and j. We apportion this drag force to the two endpoints (i, i+1) of filament i as follows: If

$\mathbf{x_{i,j}}$ is the position of the filament overlap, then we define $\lambda_{\mathbf{i,j}} = |\mathbf{x_{i,j}} - \mathbf{x_i}|/|\mathbf{x_{i+1}} - \mathbf{x_i}|$ to   111

be the fractional position of the overlap point along filament i, and we assign   112

$(1 - \lambda_{\mathbf{i,j}})\mathbf{F^{\xi}_{i,j}}$ to endpoint i and $\lambda_{\mathbf{i,j}}\mathbf{F^{\xi}_{i,j}}$ to endpoint i+1.   113

The total crosslink coupling forces on endpoints i and i+1 due to overlaps along   114

filament i can then be written: 115

$$\mathbf{F^{xl}_i} = \sum_j (1 - \lambda_{\mathbf{i,j}})\mathbf{F^{\xi}_{i,j}}$$
$$\mathbf{F^{xl}_{i+1}} = \sum_j \lambda_{\mathbf{i,j}}\mathbf{F^{\xi}_{i,j}} \tag{3}$$

where the sums are taken over all filaments j that overlap with filaments i.   116

This model assumes a linear relation between the drag force and the velocity   117

difference between attached filaments. Although non-linearities can arise through force   118

dependent detachment kinetics and/or non-linear force extension of cross-links, we   119

assume here that these non-linear effects are of second or higher order.   120

Active coupling for motor driven filament interactions   121

To add motor activity at the point of overlap between two filaments i and j ; for each   122

filament in the pair, we impose an additional force of magnitude v, directed towards its   123

(-) end (Fig. 1C):   124

$$\mathbf{F^v_i} = -v\hat{\mathbf{u}}_\mathbf{i} \tag{4}$$

and we impose an equal and opposite force on its overlapping partner. We distribute   125

these forces to filament endpoints as described above for crosslink coupling forces. Thus,   126

the total force on endpoints i and i+1 due to motor activity on overlap points between   127

filaments i and j can be written as: 128

$$\mathbf{F^{motor}_i} = v\sum_j (1 - \lambda_{\mathbf{i,j}})(\hat{\mathbf{u}}_\mathbf{i} - \hat{\mathbf{u}}_\mathbf{j})\, q_{i,j}$$
$$\mathbf{F^{motor}_{i+1}} = v\sum_j (\lambda_{\mathbf{i,j}})(\hat{\mathbf{u}}_\mathbf{i} - \hat{\mathbf{u}}_\mathbf{j})\, q_{i,j} \tag{5}$$

where j enumerates over all filaments j that overlap with filament i, and $q_{i,j}$ equals 0   129

or 1 depending on whether there is an "active" motor at this location. To model   130

dispersion of motor activity, we set $q_{i,j} = 1$ on a randomly selected subset of filament overlaps, such that $\bar{q} = \phi$, where $\bar{q}$ indicates the mean of q (Fig. 1C).

Equations of motion

To write the full equation of motion for a network of actively and passively coupled elastic filaments, we assume the low Reynold's number limit in which inertial forces can be neglected, and we equate the sum of all forces acting on each filament endpoint to zero to obtain:

$$0 = -l_s\zeta\mathbf{v_i} - \mathbf{F_i^{xl}} + \mathbf{F_i^{elas}} + \mathbf{F_i^{motor}} \tag{6}$$

where the first term represents the hydrodynamic drag on the half-filament adjoining endpoint i with respect to motion against the surrounding fluid, and ζ is the drag coefficient.

2D network formation

We used a mikado model approach [64] to initialize a minimal network of overlapping unstressed linear filaments in a rectangular 2D domain. We generate individual filaments by laying down straight lines, of length L, with random position and orientation. We define the density using the average distance between cross-links along a filament, l_c. A simple geometrical argument can then be used to derive the number of filaments filling a domain as a function of L and l_c [65]. Here, we use the approximation that the number of filaments needed to tile a rectangular domain of size $D_x \times D_y$ is $2D_xD_y/Ll_c$, and that the length density is therefore simply, $2/l_c$.

Modeling filament turnover

In living cells, actin filament assembly is governed by multiple factors that control filament nucleation, branching and elongation. Likewise filament disassembly is governed by multiple factors that promote filament severing and monomer dissociation at filament ends. Here, we implement a very simple model for filament turnover in which entire filaments appear with a fixed rate per unit area, k_{app} and disappear at a rate $k_{diss}\rho$, where ρ is a filament density (Fig. 1D). With this assumption, in the

absence of network deformation, the density of filaments will equilibrate to a steady $\qquad$ 157

state density, k_{app}/k_{diss}, with time constant $\tau_r = 1/k_{diss}$. In deforming networks, the $\qquad$ 158

density will be set by a competition between strain thinning ($\gamma > 0$) or thickening $\qquad$ 159

($\gamma < 0$), and density equilibration via turnover. To implement this model, at fixed time $\qquad$ 160

intervals $\tau_s < 0.01 \cdot \tau_r$ (i.e. 1% of the equilibration time), we selected a fraction, τ_s/τ_r, $\qquad$ 161

of existing filaments (i.e. less than 1% of the total filaments) for degradation. We then $\qquad$ 162

generated a fixed number of new unstrained filaments $k_{app}\tau_s D_x D_y$ at random positions $\qquad$ 163

and orientations within the original domain. We refer to $k_{diss} = 1/\tau_r$ as the turnover $\qquad$ 164

rate, and to τ_r as the turnover time. $\qquad$ 165

Simulation methods $\qquad$ 166

Further details regarding our simulation approach and references to our code can be $\qquad$ 167

found in the Supplementary Information (S1 Appendix A.1). Briefly, equations 1-7 $\qquad$ 168

define a coupled system of ordinary differential equations that can be written in the $\qquad$ 169

form: $\qquad$ 170

$$\mathbf{A} \cdot \dot{\mathbf{x}} = \mathbf{f}(\mathbf{x}) \tag{7}$$

where $\mathbf{x}$ is a vector of filament endpoint positions, $\dot{\mathbf{x}}$ the endpoint velocities, $\mathbf{A}$ is a $\qquad$ 171

matrix with constant coefficients that represent crosslink coupling forces between $\qquad$ 172

overlapping filaments, and $\mathbf{f}(\mathbf{x})$ represents the active (motor) and elastic forces on $\qquad$ 173

filament endpoints. We smoothed all filament interactions, force fields, and constraints $\qquad$ 174

linearly over small regions such that the equations contained no sharp discontinuities. $\qquad$ 175

We numerically integrate this system of equations to find the time evolution of the $\qquad$ 176

positions of all filament endpoints. We generate a network of filaments with random $\qquad$ 177

positions and orientations as described above within a domain of size D_x by D_y. For all $\qquad$ 178

simulations, we imposed periodic boundaries in the y-dimension. To impose an $\qquad$ 179

extensional force per unit length (2D stress) on the network, we constrained all $\qquad$ 180

filament endpoints within a fixed distance $0.05 \cdot D_x$ from the left edge of the domain to $\qquad$ 181

be non-moving, then we imposed a rightwards force on all endpoints within a distance $\qquad$ 182

$0.05 \cdot D_x$ from the right edge of the domain, such that the force per unit length of $\qquad$ 183

boundary equals the desired stress value. To simulate free contraction, we removed all $\qquad$ 184

constraints at domain boundaries; to assess buildup and maintenance of contractile   185

stress under isometric conditions, we used periodic boundary conditions in both x and y 186

dimensions.   187

Measuring stress, strain, and strain rate   188

In our 2D model, The analogue of stress is a force per unit length, which we refer to   189

simply as a stress. We measured the internal network stress at each axial position by   190

summing the axial (x) component of the tensions on all filaments intersecting that   191

position, and dividing by network height D_y. We measured the strain on an individual   192

filament as the change in filament length, divided by its equilibrium length. Then we   193

averaged this measurement over all filaments in a network to obtain an average filament   194

strain. To measure the average network strain rate, we first measured the mean velocity   195

v(X) at position X (relative to the network boundary at x = 0) to be the average   196

velocities of all filaments intersecting that position. In the cases where we measure   197

network strain or strain rates, we observed an approximately linear dependence of v(X)   198

on X; hence the strain rate is approximately uniform across the network (Fig. 2B and   199

S6 Fig). Accordingly, for each filament, we took $\frac{1}{X}\frac{dX}{dt}$ to be an estimate of the strain   200

rate on the network between x = 0 and x = X. We averaged this estimate over all   201

filaments in a domain to get an average strain rate. Finally, to estimate the cumulative   202

network strain at a given time T in the simulation, we integrated the strain rate with   203

respect to time for t = 0 to T.   204

We assigned biological plausible reference values for all parameters (See Table 1).   205

For individual analyses, we sampled the ranges of parameter values around these   206

reference values shown in S1 Table.   207

Table 1. Simulation parameters with reference values

Parameter	Symbol	Reference Value
extensional modulus	μ_e	$1nN$
compressional modulus	μ_c	$0.01nN$
cross-link drag coefficient	ξ	$1\frac{pNs}{\mu m}$
solvent drag coefficient	ζ	$0.0005\frac{nNs}{\mu m^2}$
filament length	L	$1-5\mu m$
cross-link spacing	l_c	$0.5\mu m$
active filament force	υ	$0.1nN$
active cross-link fraction	ϕ	$0.1-0.9$
domain size	$D_x \times D_y$	$20 \times 50\mu m$

Results

The goal of this study is to understand how cortical flow is shaped by the simultaneous dependencies of active stress and effective viscosity on filament turnover, crosslink drag and on "network parameters" that control filament density, elasticity and motor activity. We approach this in three steps: First, we analyze the passive deformation of a cross-linked network in response to an externally applied stress; we identify regimes in which the network response is effectively viscous and characterize the dependence of effective viscosity on network parameters and filament turnover. Second, we analyze the buildup and dissipation of active stress in cross-linked networks with active motors, as they contract against an external resistance; we identify conditions under which the network can produce sustained stress at steady state, and characterize how steady state stress depends on network parameters and filament turnover. Finally, we confirm that the dependencies of active stress and effective viscosity on network parameters and filament turnover are sufficient to predict the dynamics of networks undergoing steady state flow in response to spatial gradients of motor activity.

Filament turnover allows and tunes effectively viscous steady state flow.

Networks with passive cross-links and no filament turnover undergo three stages of deformation in response to an extensional force. To characterize the passive response of a cross-linked filament network without filament recycling, we simulated a simple uniaxial strain experiment in which we pinned the network at one end, imposed an external stress at the opposite end, and then quantified internal stress and cumulative network strain as a function of time (Fig. 1E). The typical response occurred in three qualitatively distinct phases (Fig. 2A,C). At short times the response was viscoelastic, with a rapid buildup of internal stress and a rapid ∼exponential approach to a level of cumulative strain (S1 FigA), that represents the elastic limit in the absence of cross-link slip predicted by [65]. At intermediate times, the local stress and strain rate were approximately constant across the network (Fig. 2B), and the response was effectively viscous; internal stress remained constant while the network continued to deform slowly and continuously with nearly constant strain rate (shown as

dashed line in Fig. 2C) as filaments slip past one another against the effective cross-link ₂₃₈

drag. In this regime, we can quantify effective viscosity, η_c, as the ratio of applied stress ₂₃₉

to the measured strain rate. Finally, as cumulative network strain increased, there was a ₂₄₀

corresponding decrease in filament density. As cumulative strain approached a critical ₂₄₁

value ($\sim 30\%$ for the simulation in Fig. 2), the decrease in filament density lead to ₂₄₂

decreased network connectivity, local tearing, and rapid acceleration of the network ₂₄₃

deformation (see inset in Fig. 2C). ₂₄₄

Fig 2. Networks with passive cross-links and no filament turnover undergo three stages of deformation in response to an extensional stress. **A)** Three successive time points from a simulation of a $4 \times 6.6\,\mu m$ network deforming under an applied stress of $0.005\,nN/\mu m$. Stress (tan arrows) is applied to filaments in the region indicated by the tan bar. In this and all subsequent figures, filaments are color-coded with respect to state of strain (blue = tension, red = compression). Network parameters: $L = 1\,\mu m$, $l_c = 0.3\,\mu m$, $\xi = 100\,nN \cdot s/\mu m$. **B)** Mean filament stress and velocity profiles for the network in (a) at t=88s. Note that the stress is nearly constant and the velocity is nearly linear as predicted for a viscous fluid under extension. **C)** Plots of the mean stress and cumulative network strain vs time for the simulation in (a), illustrating the three stages of deformation: (i) A fast initial deformation accompanies rapid buildup of internal network stress; (ii) after a characteristic time τ_c (indicated by vertical dotted line) the network deforms at a constant rate, i.e. with a constant effective viscosity, η_c, given by the slope of the dashed line; (iii) at long times, decrease in filament density, and loss of network connectivity, leads to material failure (see inset)

Network architecture sets the rate and timescales of deformation. To ₂₄₅

characterize how effective viscosity and the timescale for transition to effectively viscous ₂₄₆

behavior depend on network architecture and cross-link dynamics, we simulated a ₂₄₇

uniaxial stress test, holding the applied stress constant, while varying filament length L, ₂₄₈

density l_c, elastic modulus μ_e and cross link drag ξ (see S1 Table). We measured the ₂₄₉

elastic modulus, G_0, the effective viscosity, η_c, and the timescale τ_c for transition from ₂₅₀

viscoelastic to effectively viscous behavior, and compared these to theoretical predictions. ₂₅₁

We observed a transition from viscoelastic to effectively viscous deformation for the ₂₅₂

entire range of parameter values that we sampled. Our estimate of G_0 from simulation ₂₅₃

agreed well with the closed form solution $G_0 \sim \mu/l_c$ predicted by a previous theoretical ₂₅₄

model [65] for networks of semi-flexible filaments with irreversible cross-links (Fig. 3B). ₂₅₅

A simple theoretical analysis of filament networks with frictional cross link slip, ₂₅₆

operating in the intermediate viscous regime (see S1 Appendix A.2), predicted that the ₂₅₇

effective viscosity η_c should be proportional to the cross-link drag coefficient and to the ₂₅₈

Fig 3. Network architecture sets the rate and timescales of deformation. **(a-c)** Comparison of predicted and simulated values for: **A)** the bulk elastic modulus G_0, **B)** the effective viscosity η_c and **C)** the timescale for transition from viscoelastic to viscous behavior τ_c, given by the ratio of the bulk elastic modulus G_0 to effective viscosity, η_c. Dotted lines indicates the relationships predicted by theory.

square of the number of cross-links per filament:

$$\eta_c = 4\pi\xi \left(\frac{L}{l_c} - 1\right)^2 \tag{8}$$

As shown in Fig. 3B, our simulations agree well with this prediction for a large range of sampled network parameters. Finally, for many linear viscoelastic materials, the ratio of effective viscosity to the elastic modulus η_c/G_0 sets the timescale for transition from elastic to viscous behavior [66]. Combining our approximations for G_0 and η_c, we predict a transition time, $\tau_c \approx L^2\xi/l_c\mu$. Measuring the time at which the strain rate became nearly constant (i.e. $\gamma \sim t^n$ with $n > 0.8$) yields an estimate of τ_c that agrees well with this prediction over the entire range of sampled parameters (Fig. 3C). Thus the passive response of filament networks with frictional cross link drag is well-described on short (viscoelastic) to intermediate (viscous) timescales by an elastic modulus G_0, an effective viscosity η_c, and a transition timescale τ_c, with well-defined dependencies on network parameters. On longer timescales, the cumulative decrease in filament density leads to material failure.

Filament turnover allows sustained large-scale viscous flow and defines two distinct flow regimes. To characterize how filament turnover shapes the passive network response to an applied force, we introduced a simple form of turnover in which entire filaments disappear at a rate $k_{diss}\rho$, where ρ is the filament density, and new unstrained filaments appear with a fixed rate per unit area, k_{ass}. Absent deformation, filament density will equilibrate to a steady state value, $\rho_0 = k_{ass}/k_{diss}$, with time constant $\tau_r = 1/k_{diss}$. However, in networks deforming under extensional stress, density will be shaped by both turnover and network extension, and changes in density will feed back on deformation through the density-dependence of effective viscosity (Fig. 3B).

To examine the consequences of these effects, we simulated a uniaxial stress test for different values of τ_r, while holding ρ_0 and all other parameters fixed (Fig. 4A-C). For

large τ_r, network extension was accompanied by a continuous decrease in filament $\quad$ 283

density, leading ultimately to loss of connectivity and material failure (S2 Fig). For $\quad$ 284

lower values of τ_r, the network approached a steady state characterized by continuous $\quad$ 285

extension at a constant density and strain rate (Fig. 4B, S2 Fig). Analysis of a simple $\quad$ 286

coarse-grained model (S1 Appendix A.3) revealed how this behavior emerges from a $\quad$ 287

competition between density equilibration via turnover and the decrease in density, and $\quad$ 288

thus effective viscosity, during extension. For τ_r greater than a critical lifetime τ_{crit}, the $\quad$ 289

latter effect dominates, leading to a runaway decrease in filament density, loss of $\quad$ 290

connectivity and material failure. For $\tau_r < \tau_{crit}$, the coarse-grained model predicts the $\quad$ 291

existence of a dynamically stable steady state characterized by continuous extension at $\quad$ 292

constant density and strain rate (S1 Appendix A.3), as observed in our simulations (S3 $\quad$ 293

Fig). $\quad$ 294

Fig 4. Filament turnover defines two regimes of effectively viscous flow. **A)**
Comparison of $20 \times 12\mu m$ networks under $0.001\ nN/\mu m$ extensional stress without
(top) and with (bottom) filament turnover. Both images are taken when the networks
had reached a net strain of 0.04. For clarity, filaments that leave the domain of applied
stress are greyed out. **B)** Plots of strain vs time for identical networks with different
rates of filament turnover. Network parameters: $L = 5\ \mu m$, $l_c = 0.5\ \mu m$,
$\xi = 10\ nN \cdot s/\mu m$. **C)** Plot of effective viscosity vs turnover time derived from the
simulations shown in panel b. Square dot is the $\tau_r = \infty$ condition. **D)** Plot of
normalized effective viscosity (η/η_c) vs normalized turnover time (τ_r/τ_c) for a large
range of network parameters and turnover times. For $tau_r \ll \tau_c$, the viscosity of the
network becomes dependent on turnover time. Red dashed line indicates the
approximation given in equation 9 for $m = 3/4$.

For $\tau_r < \tau_{crit}$, we observed two distinct steady state flow regimes (Fig. 4B,C). For $\quad$ 295

intermediate values of τ_r, effective viscosity remains constant with decreasing τ_r. $\quad$ 296

However, below a certain value of τ_r ($\approx 10^3$ for the parameters used in Fig. 4C), $\quad$ 297

effective viscosity decreased monotonically with further decreases in τ_r. To understand $\quad$ 298

what sets the timescale for transition between these two regimes, we measured effective $\quad$ 299

viscosity at steady steady for a wide range of network parameters (L, μ, l_c), crosslink $\quad$ 300

drags (ξ) and filament turnover times (Fig. 4D). Strikingly, when we plotted the $\quad$ 301

normalized effective viscosity η_r/η_c vs a normalized turnover time τ_r/τ_c for all $\quad$ 302

parameter values, the data collapsed onto a single curve, with a transition at $\tau_r \approx \tau_c$ $\quad$ 303

between an intermediate turnover regime in which effective viscosity is independent of $\quad$ 304

τ_r and an high turnover regime in which effective viscosity falls monotonically with $\quad$ 305

decreasing τ_r/τ_c (Fig. 4D).

This biphasic dependence of effective viscosity on filament turnover can be understood intuitively as follows: As new filaments are born, they become progressively stressed as they stretch and reorient under local influence of surrounding filaments, eventually reaching an elastic limit where their contribution to resisting network deformation is determined by effective crosslink drag. The time to reach this limit is about the same as the time, τ_c, for an entire network of initially unstrained filaments to reach an elastic limit during the initial viscoelastic response to uniaxial stress, as shown in Fig. 2b. For $\tau_r < \tau_c$, individual filaments do not have time, on average, to reach the elastic limit before turning over; thus the deformation rate is determined by the elastic resistance of partially strained filaments, which increases with lifetime up to $\tau_r = \tau_c$. For $\tau_r > \tau_c$, the deformation rate is largely determined by cross-link resistance to sliding of maximally strained filaments, and the effective viscosity is insensitive to further increase in τ_r.

These results complement and extend a previous computational study of irreversibly cross-linked networks of treadmilling filaments deforming under extensional stress [67]. Kim et al identified two regimes of effectively viscous deformation: a "stress-dependent" regime in which filaments turnover before they become strained to an elastic limit and deformation rate is proportional to both applied stress and turnover rate; and a "stress-independent" regime in which filaments reach an elastic limit before turning over and deformation rate depends only on the turnover rate. The fast and intermediate turnover regimes that we observe here correspond to the stress-dependent and independent regimes described by Kim et al, but with a key difference. Without filament turnover, Kim et al's model predicts that a network cannot deform beyond its elastic limit. In contrast, our model predicts viscous flow at low turnover, governed by an effective viscosity that is set by cross-link density and effective drag. Thus our model provides a self-consistent framework for understanding how crosslink unbinding and filament turnover contribute separately to viscous flow and connects these contributions directly to previous theoretical descriptions of cross-linked networks of semi-flexible filaments.

In summary, our simulations predict that filament turnover allows networks to undergo viscous deformation indefinitely, without loss of connectivity or material failure,

over a wide range of different effective viscosities and deformation rates. For $\tau_r < \tau_{crit}$, 338

this behavior can be summarized by an equation of the form: 339

$$\eta = \frac{\eta_c}{1 + (\tau_c/\tau_r)^m} \tag{9}$$

For $\tau_r \gg \tau_c$, $\eta \approx \eta_c$: effective viscosity depends on crosslink density and effective 340

crosslink drag, independent of changes in recycling rate. For $\tau_r \ll \tau_c$, effective viscosity 341

is governed by the level of elastic stress on network filaments, and becomes strongly 342

dependent on filament lifetime: $\eta \sim \eta_c(\tau_r/\tau_c)^m$. The origins of the $m = 3/4$ scaling 343

remains unclear (see Discussion). 344

Filament turnover allows persistent stress buildup in active 345
networks 346

In the absence of filament turnover, active networks with free boundaries 347

contract and then stall against passive resistance to network compression. 348

Previous work [38, 40, 68] identifies asymmetric filament compliance and spatial 349

heterogeneity in motor activity as minimal requirements for macroscopic contraction of 350

disordered networks. To confirm that our simple implementation of these two 351

requirements (see Models section) is sufficient for macroscopic contraction, we simulated 352

active networks that are unconstrained by external attachments, varying filament 353

length, density, crosslink drag and motor activity. We observed qualitatively similar 354

results for all choices of parameter values: Turning on motor activity in an initially 355

unstrained network induced rapid initial contraction, followed by a slower buildup of 356

compressive stress (and strain) on individual filaments, and an ~exponential approach 357

to stall (Fig. 5). The time to stall, τ_s, scaled as $L\xi/\upsilon$ (S3 FigA). On even longer 358

timescales, polarity sorting of individual filaments, as previously described [42, 44–46] 359

lead to network expansion (see S2 Video). 360

During the rapid initial contraction, the increase in network strain closely matched 361

the increase in mean compressive strain on individual filaments Fig. 5B, as predicted 362

theoretically [38, 39] and observed experimentally [40]. Contraction required asymmetric 363

filament compliance and spatial heterogeneity of motor activity ($\mu_e/\mu_c > 1$, $\phi < 1$, S3 364

FigB). Thus our model captures a minimal mechanism for bulk contractility in 365

Fig 5. In the absence of filament turnover, active networks with free boundaries contract and then stall against passive resistance to network compression. **A)** Simulation of an active network with free boundaries. Colors represent strain on individual filaments as in previous figures. Note the buildup of compressive strain as contraction approaches stall between 100 s and 150 s. Network parameters: $L = 5\,\mu m$, $l_c = 0.3\,\mu m$, $\xi = 1\,nN \cdot s/\mu m$, $v = 0.1\,nN$. **B)** Plots showing time evolution of total network strain (black) and the average extensional (blue) or compressive (red) strain on individual filaments. **C)** Plots showing time evolution of total (black) extensional (blue) or compressive (red) stress. Note that extensional and compressive stress remain balanced as compressive resistance builds during network contraction.

disordered networks through asymmetric filament compliance and dispersion of motor activity.

Active networks cannot sustain stress against a fixed boundary in the absence of filament turnover. During cortical flow, regions with high motor activity contract against a passive resistance from neighboring regions with lower motor activity. To understand how the active stresses that drive cortical flow are shaped by motor activity and network remodeling, we analyzed the buildup and maintenance of contractile stress in active networks contracting against a rigid boundary. We simulated active networks contracting from an initially unstressed state against a fixed boundary (Fig. 6A,B), and monitored the time evolution of mean extensional (blue), compressional (red) and total (black) stress on network filaments (Fig. 6C,D). We focused initially on the scenario in which there is no, or very slow, filament turnover, sampling a range of parameter values controlling filament length and density, motor activity, and crosslink drag.

We observed a similar behavior in all cases: total stress built rapidly to a peak value σ_m, and then decayed towards zero (Fig. 6C,D). The rapid initial increase in total stress was determined largely by the rapid buildup of extensional stress (Fig. 6C,D) on a subset of network filaments (Fig. 6A,B $t = 10s$). The subsequent decay involved two different forms of local remodeling: under some conditions, e.g. for higher motor activity (e.g. Fig. 6A,C), the decay was associated with rapid local tearing and fragmentation, leading to global loss of network connectivity as described previously both in simulations [48] and *in vitro* experiments [41]. However, for many parameters, (e.g. for higher motor activity as in Fig. 6B,D), the decay in stress occurred with little or no loss of global connectivity. Instead, local filament rearrangements changed the

Fig 6. In the absence of filament turnover, active networks cannot sustain continuous stress against a fixed boundary. **A)** Simulation of an active network with fixed boundaries. Rearrangement of network filaments by motor activity leads to rapid loss of network connectivity. Network parameters: $L = 5\,\mu m$, $l_c = 0.3\,\mu m$, $\xi = 1\,nN \cdot s/\mu m$, $\upsilon = 1\,nN$. **B)** Simulation of the same network, with the same parameter values, except with ten-fold lower motor activity $\upsilon = 0.1\,nN$. In this case, connectivity is preserved, but there is a progressive buildup of compressive strain on individual filaments. **C)** Plots of total network stress and the average extensional (blue) and compressive (red) stress on individual filaments for the simulation shown in (a). Rapid buildup of extensional stress allows the network transiently to exert force on its boundary, but this force is rapidly dissipated and this dissipation is associated with a breakdown in network connectivity. **D)** Plots of total network stress and the average extensional (blue) and compressive (red) stress on individual filaments for the simulation shown in (b). Rapid buildup of extensional stress allows the network transiently to exert force on its boundary, but this force is dissipated at longer times as decreasing extensional stress and increasing compressive stress approach balance. Note the different time scales used for plots and subplots in **C)** and **D)** to emphasize the similar timescales for force buildup, but very different timescales for force dissipation.

balance of extensile vs compressive forces along individual filaments, leading to a slow $\quad$ 390

decrease in the average extensional stress, and a correspondingly slow increase in the $\quad$ 391

compressional stress, on individual filaments (see Fig. 6D). $\quad$ 392

Combining dimensional analysis with trial and error, we were able to find empirical $\quad$ 393

scaling relationships describing the dependence of maximum stress σ_m and the time to $\quad$ 394

reach maximum stress τ_m on network parameters and effective crosslink drag $\quad$ 395

$(\sigma_m \sim \sqrt{\mu_e \upsilon}/l_c,\ \tau_m \sim L\xi/\sqrt{\mu_e \upsilon}$, S3 FigC,D). Although these relationships should be $\quad$ 396

taken with a grain of salt, they are reasonably consistent with our simple intuition that $\quad$ 397

the peak stress should increase with motor force (υ), extensional modulus (μ_e) and $\quad$ 398

filament density ($1/l_c$), and the time to reach peak stress should increase with crosslink $\quad$ 399

drag (ξ) and decrease with motor force (υ) and extensional modulus (μ_e). $\quad$ 400

Filament turnover allows active networks to exert sustained stress on a $\quad$ 401

fixed boundary. Regardless of the exact scaling dependencies of σ_m and τ_m on $\quad$ 402

network parameters, these results reveal a fundamental limit on the ability of active $\quad$ 403

networks to sustain force against an external resistance in the absence of filament $\quad$ 404

turnover. To understand how this limit can be overcome by filament turnover, we $\quad$ 405

simulated networks contracting against a fixed boundary from an initially unstressed $\quad$ 406

state, for increasing rates of filament turnover (decreasing τ_r), while holding all other $\quad$ 407

parameter values fixed (Fig. 7A-C). While the peak stress decreased monotonically with $\quad$ 408

decreasing τ_r, the steady state stress showed a biphasic response, increasing initially   409

with decreasing τ_r, and then falling off as $\tau_r \to 0$. We observed a biphasic response   410

regardless of how stress decays in the absence of turnover, i.e. whether decay involves   411

loss of network connectivity, or local remodeling without loss of connectivity, or both   412

(S4 Fig and not shown). Significantly, when we plot normalized steady state stress   413

(σ/σ_m) vs normalized recycling time (τ_r/τ_m) for a wide range of network parameters,   414

the data collapse onto a single biphasic response curve, with a peak near $\tau_r/\tau_m = 1$ 415

(Fig. 7D). In particular, for $\tau_r < \tau_m$, the scaled data collapsed tightly onto a single   416

curve representing a linear increase in steady state stress with increasing τ_r. For   417

$\tau_r > \tau_m$, the scaling was less consistent, although the trend towards a monotonic   418

decrease with increasing τ_r was clear. These results reveal that filament turnover can   419

"rescue" the dissipation of active stress during isometric contraction due to network   420

remodeling, and they show that, for a given choice of network parameters, there is an   421

optimal choice of filament lifetime that maximizes steady state stress.   422

Fig 7. Filament turnover allows active networks to exert sustained stress on a fixed boundary. **A)** Snapshots from simulations of active networks with fixed boundaries and different rates of filament turnover. All other parameter values are the same as in Fig. 6A. Note the significant buildup of compressive strain and significant remodeling for longer, but not shorter, turnover times. **B)** Plots of net stress exerted by the network on its boundaries for different recycling times; for long-lived filaments, stress is built rapidly, but then dissipates. Decreasing filament lifetimes reduces stress dissipation by replacing compressed with uncompressed filaments, allowing higher levels of steady state stress; for very short lifetimes, stress is reduced, because individual filaments do not have time to build stress before turning over. **C)** Plots of ≈steady state stress estimated from the simulations in **B)** vs turnover time. **D)** Plot of normalized steady state stress vs normalized recycling time for a wide range of network parameters and turnover times. Steady state stress is normalized by the predicted maximum stress σ_m achieved in the absence of filament turnover. Turnover time is normalized by the predicted time to achieve maximum stress τ_m, in the absence of filament turnover. Predictions for σ_m and τ_m were obtained from the phenomenological scaling relations shown in (Fig. C,D). Dashed blue line indicates the approximation given in equation 10 for $n = 1$.

We can understand the biphasic dependence of steady state stress on filament   423

lifetime using the same reasoning applied to the case of passive flow: During steady   424

state contraction, the average filament should build and dissipate active stress on   425

approximately the same schedule as an entire network contracting from an initially   426

unstressed state (Fig. 7B). Therefore for $\tau_r < \tau_m$, increasing lifetime should increase the   427

mean stress contributed by each filament. For $\tau_r > \tau_m$, further increases in lifetime   428

should begin to reduce the mean stress contribution. Directly comparing the

time-dependent buildup and dissipation of stress in the absence of turnover, with the

dependence of steady state stress on τ_r, supports this interpretation (S5 Fig)

As for the passive response (i.e. Equation 9), we can describe this biphasic

dependence phenomenologically with an equation of the form:

$$\sigma_{ss} = \frac{\sigma_m}{(\tau_r/\tau_m)^n + \tau_m/\tau_r} \tag{10}$$

where the origins of the exponent n remain unclear.

Filament turnover tunes the balance between active stress buildup and viscous stress relaxation to generate flows

Thus far, we have considered independently how network remodeling controls the

passive response to an external stress, or the steady state stress produced by active

contraction against an external resistance. We now consider how these two forms of

dependence will combine to shape steady state flow produced by spatial gradients of

motor activity. We consider a simple scenario in which a network is pinned on either

side and motor activity is continuously patterned such that the right half network has

uniformly high levels of motor activity (controlled by v, with $\psi = 0.5$), while the left

half network has none. Under these conditions, the right half network will contract

continuously against a passive resistance from the left half network. Because of

asymmetric filament compliance, the internal resistance of the right half network to

active compression should be negligible compared to the external resistance of the left

half network. Thus the steady state flow will be described by:

$$\dot{\gamma} = \frac{\sigma_{ss}}{\eta} \tag{11}$$

where σ_{ss} is the active stress generated by the right half-network (less the internal

resistance to filament compression), η is the effective viscosity of the left half network

and strain rate $\dot{\gamma}$ is measured in the left half-network. Note that strain rate can be

related to the steady state flow velocity v at the boundary between right and left halves

through $v = \dot{\gamma}Dx$. Therefore, we can understand the dependence of flow speed on

filament turnover and other parameters using the approximate relationships summarized   454

by equations 9 and 10 for η and σ_{ss}. As shown in Fig. 8, there are two qualitatively   455

distinct possibilities for the dependence of strain rate on τ_r, depending on the relative   456

magnitudes of τ_m and τ_c. In both cases, for fast enough turnover ($\tau_r < \min(\tau_m, \tau_c)$), we   457

expect weak dependence of strain rate on τ_r ($\dot{\gamma} \sim \tau_r^{1/4}$). For all parameter values that   458

we sampled in this study (which were chosen to lie in a physiological range), $\tau_m > \tau_c$.   459

Therefore we predict the dependence of steady state strain rate on τ_r shown in Fig. 8A.   460

Fig 8. Filament recycling tunes the magnitudes of both effective viscosity and steady
state stress. **A)** Dependence of steady state stress, effective viscosity, and resulting
strain rate on recycling time τ_r under the condition $\tau_m < \tau_c$. **B)** Same as a) but for
$\tau_c < \tau_m$. **C)** State space of flow rate dependence relative to the two relaxation
timescales, τ_r and τ_c normalized by the stress buildup timescale, τ_m.

To confirm this prediction, we simulated the simple scenario described above for a   461

range of values of τ_r, with all other parameter values initially fixed at their reference   462

values (See Table 1). As expected, we observed a sharp dependence of steady state flow   463

speeds on filament recycling rate (Fig. 9B,C). For very long recycling times,   464

($\tau_r = 1000s$, dark blue line), there was a rapid initial deformation (contraction of the   465

active domain and dilation of the passive domain), followed by a slow approach to a   466

steady state flow characterized by slow contraction of the right half-domain and a   467

matching dilation of the left half-domain (see S6 Fig). However, with decreasing values   468

of τ_r, steady state flow speeds increased steadily, before reaching an approximate   469

plateau on which flow speeds varied by less than 15 % over more than two decades of   470

variation in τ_r (Fig. 9C). For the reference values of model parameters, the steady state   471

strain rates ($\approx 2x10^{-4}/sec$) were somewhat lower than, but comparable to those   472

measured during steady state flows in *C. elegans* ($\approx 1.3x10^{-3}/sec$) [?].   473

We repeated these simulations for a wider range of parameter values, and saw similar   474

dependence of $\dot{\gamma}$ on τ_r in all cases. Using equation 9 with $\tau_r < \tau_c$ and equation 10 with   475

$\tau_r < \tau_m$, and the theoretical or empirical scaling relationships found above for η_c, τ_c,   476

σ_m and τ_m, we predict a simple scaling relationship for $\dot{\gamma}$ (for small τ_r):   477

$$\dot{\gamma} = \frac{\upsilon}{\xi L}\left(\tau_r\right)^{1/4} \tag{12}$$

Indeed, when we plot the steady state measurements of $\dot{\gamma}$, normalized by $\upsilon/\xi L$, for   478

Fig 9. Filament recycling allows sustained flows in networks with non-isotropic activity. **A)** Example simulations of non-isotropic networks with long ($\tau_r = 1000$) and short ($\tau_r = 33$) recycling timescales. In these networks the left half of the network is passive while the right half is active. Network parameters are same as in Fig.s 6 and 7. Importantly, in all simulations $\tau_m < \tau_c$. **B)** Graph of strain for identical networks with varying recycling timescales. With long recycling times, the network stalls; reducing the recycling timescale allows the network to persist in its deformation. However, for the shortest recycling timescales, the steady state strain begins to slowly return to 0 net motion. Measurements are based on the passive side of the network. **C)** Steady state strain rates for the networks in (b). **D)** Graph of network's long-term strain rate as a function of recycling timescale. Dashed line is form of dependence predicted by the theoretical arguments shown in Fig. 8.

all parameter values, the data collapse onto a single curve for small τ_r. Thus. our   479
simulations identify a flow regime, characterized by sufficiently fast filament turnover, in   480
which the steady state flow speed is buffered against variation in turnover, and has a   481
relatively simple dependence on other network parameters.   482

Discussion   483

Cortical flows arise through a dynamic interplay of force production and dissipation   484
within cross-linked actomyosin networks. Here we combined computer simulations with   485
simple theoretical analysis to explore how this interplay depends on motor activity,   486
crosslink dynamics, network architecture and filament turnover. Our results reveal two   487
essential requirements for filament turnover during cortical flow: (a) to allow the   488
continuous relaxation of elastic resistance without catastrophic loss of network   489
connectivity and (b) to prevent the dissipation of active stress through local network   490
rearrangements. We find that biphasic dependencies of active stress and passive   491
relaxation on filament lifetime define multiple modes of steady state flow with distinct   492
dependencies on network parameters and filament turnover.   493

We identify two distinct modes of passive response to uniaxial stress: a low turnover   494
mode in which filaments strain to an elastic limit before turning over, and effective   495
viscosity depends on crosslink density and effective crosslink friction, and a high   496
turnover mode in which filaments turn over before reaching an elastic limit and effective   497
viscosity is proportional to elastic resistance and $\approx$ proportional to filament lifetime.   498
We note that the weakly sub-linear dependence of effective viscosity on filament lifetime   499

that we observe in the high turnover regime may simply reflect a failure to capture very   500
local modes of filament deformation, since a previous study [67] in which filaments were   501
represented as connected chains of smaller segments predicted linear dependence of   502
effective viscosity on filament lifetime. While previous studies have emphasized   503
individual roles for cross-link unbinding or filament turnover in stress   504
relaxation [7, 13, 22], here we have capture their distinct contributions within a single   505
self-consistent modeling framework.   506

Our simulations confirm the theoretical prediction [38, 40, 68] that spatial   507
heterogeneity of motor activity and asymmetric filament compliance are sufficient to   508
support macroscopic contraction of unconstrained networks. However, under isometric   509
conditions, and without filament turnover, our simulations predict that active stress   510
cannot be sustained. On short timescales, motor forces drive local buildup of   511
extensional stress, but on longer timescales, active local filament rearrangements drive   512
local changes in connectivity that lead, invariably, to a decay in active stress. Under   513
some conditions, contractile forces drive networks towards a critically connected state,   514
leading to tearing and fragmentation, as previously described [41, 48]. However, we find   515
that stress decay can also occur without any global loss of connectivity, through a   516
gradual decrease in extensile force and a gradual increase in compressive force along   517
individual filaments. Our results suggest that when filaments can slide relative to one   518
another, the motor forces that produce active stress will inevitably lead to local changes   519
in connectivity that decrease active stress. Thus for contractile networks to maintain   520
isometric tension on long timescales, they must either form stable crosslinks to prevent   521
filament rearrangements, or they must continuously recycle network filaments (or active   522
motors) to renew the local potential for production of active stress.   523

Indeed, our simulations predict that filament turnover is sufficient for maintenance of   524
active stress. As in the passive case, they predict biphasic dependence of steady state   525
stress on filament turnover: For short-lived filaments ($\tau_r < \tau_m$), steady state stress   526
increases linearly with filament lifetime because filaments have more time to build   527
towards peak extensional stress before turning over. For longer loved filaments   528
($\tau_r > \tau_m$), steady state stress decreases monotonically with filament lifetime because   529

local rearrangements decrease the mean contributions of longer lived filaments. These 530 findings imply that for cortical networks that sustain contractile stress under 531 approximately isometric conditions, tuning filament turnover can control the level of 532 active stress, and there will be an optimal turnover rate that maximizes the stress, all 533 other things equal. This may be important, for example in early development, where 534 contractile forces produced by cortical actomyosin networks maintain, or drive slow 535 changes in, cell shape and tissue geometry [7, 69]. 536

For cortical networks that undergo steady state flows driven by spatial gradients of 537 motor activity, our simulations predict that the biphasic dependencies of steady state 538 stress and effective viscosity on filament lifetime define multiple regimes of steady state 539 flow, characterized by different dependencies on filament turnover (and other network 540 parameters). In particular, the ∼linear dependencies of steady state stress and effective 541 viscosity on filament lifetime for short-lived filaments define a fast turnover regime in 542 which steady state flow speeds are buffered against variations in filament lifetime, and 543 are predicted to depend in a simple way on motor activity and crosslink resistance. 544 Measurements of F-actin turnover times in cells that undergo cortical flow [32, 70–74] 545 suggests that they may indeed operate in this fast turnover regime, and recent studies 546 in *C. elegans* embryos suggests that cortical flow speeds are surprisingly insensitive to 547 depletion of factors (ADF/Cofilin) that govern filament turnover [11], consistent with 548 our model's predictions. Stronger tests of our model's predictions will require more 549 systematic analyses of how flow speeds vary with filament and crosslink densities, motor 550 activities, and filament lifetimes. 551

Supporting Information 552

S1 Appendix. Code Reference and Supplementary Methods A.1) Reference 553 to simulation and analysis code. **A.2)** Derivation of effective viscosity. **A.3)** Derivation 554 of critical turnover timescale for steady state flow 555

S1 Table. Parameter values. List of parameter values used for each set of 556 simulations. 557

S1 Fig.   Fast viscoelastic response to extensional stress. Plots of normalized cumulative strain vs time during the elastic phase of deformation in passive networks under extensional stress. Measured strain is normalized by the equilibrium strain predicted for a network of elastic filaments without crosslink slip $\gamma_{eq} = \sigma/G_0 = \sigma/(2\mu/l_c)$.

S2 Fig.   Filament turnover limits density decrease under extensional strain and allows continuous flow without material failure. A) Plots of cumulative strain vs time for different turnover times (see inset in (b)). Note the increase in strain rates with decreasing turnover time. **B)** Plots of filament length density vs time for different turnover times τ_r. For long to intermediate τ_r, simulations predict an $\approx$ linear decrease in length density with time, at a rate that decreases with decreasing τ_r, leading ultimately to loss of connectivity and material failure. For lower τ_r, length densities approach steady state values at longer times. These results match the predictions of the corase grained analysis in Appendix section A.3.

S3 Fig.   Mechanical properties of active networks. A) Time for freely contracting networks to reach maximum cumulative strain, τ_s, scales with $L\xi/\upsilon$. **B)** Free contraction requires asymmetric filament compliance, and total cumulative network strain increases with the applied myosin force υ. Note that the maximum contraction approaches an asymptotic limit as the stiffness asymmetry approaches a ratio of ~ 100. **C)** Maximum stress achieved during isometric contraction, σ_m, scales approximately with $\sqrt{\mu_e \upsilon}/l_c$. **D)** Time to reach max stress during isometric contraction scales approximately with $L\xi/\sqrt{\mu_e \upsilon}$. Scalings for τ_s, σ_m and τ_m were determined empirically by trial and error, guided by dimensional analysis.

S4 Fig.   Filament turnover prevents loss of connectivity and local tearing of active networks. A) An active network undergoing large scale deformations due to active filament rearrangements. **B)** The same network as in (A) but with a shorter filament turnover time. **C)** Plots of internal stress vs time for the network in (A). **D)** Plots of internal stress vs time for the network in (B).

S5 Fig.   Bimodal dependence on turnover time matches bimodal buildup
and dissipation of stress in the absence of turnover. A) Bimodal buildup of
stress in a network with very slow turnover ($\tau_r = 1000s$). **B)** Steady state stress for
networks with same parameters as in (a), but for a range of filament turnover times.

S6 Fig.   Dynamics of steady state flow. Plots of stress and cumulative strain vs
position for networks in which motor activity is limited to the right-half domain and
filament turnover time is either **A)** $\tau_r = 10000$ or **B)** $\tau_r = 10s$. Blue indicates average
filament velocity while orange represents average network stress, measured as described
in the main text.

S1 Video.   Extensional strain in passive networks. Movie of simulation setup
shown in Fig. 2. Colors are the same as in figure.

S2 Video.   Active networks contracting with free boundaries. Movie of
simulation setup shown in Fig. 5. Colors are the same as in figure.

Acknowledgments

References

1. Bray D, White J. Cortical flow in animal cells. Science. 1988;239(4842):883–888.
   Available from:
   http://www.sciencemag.org/content/239/4842/883.abstract.

2. Hird SN, White JG. Cortical and cytoplasmic flow polarity in early embryonic
   cells of Caenorhabditis elegans. The Journal of Cell Biology.
   1993;121(6):1343–1355. Available from:
   http://jcb.rupress.org/content/121/6/1343.abstract.

3. Benink HA, Mandato CA, Bement WM. Analysis of Cortical Flow Models In
   Vivo. Molecular Biology of the Cell. 2000 08;11(8):2553–2563. Available from:
   http://www.ncbi.nlm.nih.gov/pmc/articles/PMC14939/.

4. Wilson CA, Tsuchida MA, Allen GM, Barnhart EL, Applegate KT, Yam PT, et al. Myosin II contributes to cell-scale actin network treadmilling through network disassembly. Nature. 2010 05;465(7296):373–377. Available from: `http://dx.doi.org/10.1038/nature08994`.

5. Rauzi M, Lenne PF, Lecuit T. Planar polarized actomyosin contractile flows control epithelial junction remodelling. Nature. 2010 Dec;468(7327):1110–1114. Available from: `http://dx.doi.org/10.1038/nature09566`.

6. Munro E, Nance J, Priess JR. Cortical Flows Powered by Asymmetrical Contraction Transport {PAR} Proteins to Establish and Maintain Anterior-Posterior Polarity in the Early C. elegans Embryo. Developmental Cell. 2004;7(3):413 – 424. Available from: `http://www.sciencedirect.com/science/article/pii/S153458070400276X`.

7. Salbreux G, Charras G, Paluch E. Actin cortex mechanics and cellular morphogenesis. Trends in Cell Biology. 2012;22(10):536 – 545. Available from: `http://www.sciencedirect.com/science/article/pii/S0962892412001110`.

8. Murrell M, Oakes PW, Lenz M, Gardel ML. Forcing cells into shape: the mechanics of actomyosin contractility. Nat Rev Mol Cell Biol. 2015 08;16(8):486–498. Available from: `http://dx.doi.org/10.1038/nrm4012`.

9. Bendix PM, Koenderink GH, Cuvelier D, Dogic Z, Koeleman BN, Brieher WM, et al. A Quantitative Analysis of Contractility in Active Cytoskeletal Protein Networks. Biophysical Journal. 2008;94(8):3126 – 3136. Available from: `http://www.sciencedirect.com/science/article/pii/S0006349508704697`.

10. Janson LW, Kolega J, Taylor DL. Modulation of contraction by gelation/solation in a reconstituted motile model. The Journal of Cell Biology. 1991;114(5):1005–1015. Available from: `http://jcb.rupress.org/content/114/5/1005`.

11. Mayer M, Depken M, Bois JS, Julicher F, Grill SW. Anisotropies in cortical tension reveal the physical basis of polarizing cortical flows. Nature. 2010 09;467(7315):617–621. Available from: `http://dx.doi.org/10.1038/nature09376`.

12. Bois JS, Jülicher F, Grill SW. Pattern Formation in Active Fluids. Phys Rev Lett. 2011 Jan;106:028103. Available from: http://link.aps.org/doi/10.1103/PhysRevLett.106.028103.

13. De La Cruz EM, Gardel ML. Actin Mechanics and Fragmentation. Journal of Biological Chemistry. 2015 07;290(28):17137–17144. Available from: http://www.jbc.org/content/290/28/17137. abstractN2-Cellphysiologicalprocessesrequiretheregulationandcoordinationof Herewereviewrecentadvancesinunderstandingthemechanicalpropertiesandstabili lengthscales. Wediscusshowforcescaninfluencelocalbiochemicalinteractions, resultingintheformationofmechanicallysensitivedynamicsteadystates. Understandingtheregulationofsuchforce-activatedchemistriesanddynamicsteady

14. Turlier H, Audoly B, Prost J, Joanny JF. Furrow Constriction in Animal Cell Cytokinesis. Biophysical Journal. 2014;106(1):114 – 123. Available from: http://www.sciencedirect.com/science/article/pii/S0006349513012447.

15. Salbreux G, Prost J, Joanny JF. Hydrodynamics of Cellular Cortical Flows and the Formation of Contractile Rings. Phys Rev Lett. 2009 Jul;103:058102. Available from: http://link.aps.org/doi/10.1103/PhysRevLett.103.058102.

16. Keren K, Yam PT, Kinkhabwala A, Mogilner A, Theriot JA. Intracellular fluid flow in rapidly moving cells. Nat Cell Biol. 2009 10;11(10):1219–1224. Available from: http://dx.doi.org/10.1038/ncb1965.

17. Marchetti MC, Joanny JF, Ramaswamy S, Liverpool TB, Prost J, Rao M, et al. Hydrodynamics of soft active matter. Rev Mod Phys. 2013 Jul;85:1143–1189. Available from: http://link.aps.org/doi/10.1103/RevModPhys.85.1143.

18. Behrndt M, Salbreux G, Campinho P, Hauschild R, Oswald F, Roensch J, et al. Forces Driving Epithelial Spreading in Zebrafish Gastrulation. Science. 2012;338(6104):257–260. Available from: http://science.sciencemag.org/content/338/6104/257.

19. Hochmuth RM. Micropipette aspiration of living cells. Journal of Biomechanics. 2000;33(1):15 – 22. Available from: http://www.sciencedirect.com/science/article/pii/S002192909900175X.

20. Evans E, Yeung A. Apparent viscosity and cortical tension of blood granulocytes determined by micropipet aspiration. Biophysical Journal. 1989 07;56(1):151–160. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1280460/.

21. Bausch AR, Ziemann F, Boulbitch AA, Jacobson K, Sackmann E. Local Measurements of Viscoelastic Parameters of Adherent Cell Surfaces by Magnetic Bead Microrheometry. Biophysical Journal. 1998;75(4):2038 – 2049. Available from: http://www.sciencedirect.com/science/article/pii/S0006349598776465.

22. De La Cruz EM. How cofilin severs an actin filament. Biophysical reviews. 2009 05;1(2):51–59. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2917815/.

23. Broedersz CP, Depken M, Yao NY, Pollak MR, Weitz DA, MacKintosh FC. Cross-Link-Governed Dynamics of Biopolymer Networks. Phys Rev Lett. 2010 Nov;105:238101. Available from: http://link.aps.org/doi/10.1103/PhysRevLett.105.238101.

24. Müller KW, Bruinsma RF, Lieleg O, Bausch AR, Wall WA, Levine AJ. Rheology of Semiflexible Bundle Networks with Transient Linkers. Phys Rev Lett. 2014 Jun;112:238102. Available from: http://link.aps.org/doi/10.1103/PhysRevLett.112.238102.

25. Kim T, Hwang W, Kamm RD. Dynamic Role of Cross-Linking Proteins in Actin Rheology. Biophysical Journal. 2011 10;101(7):1597–1603. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3183755/.

26. Lieleg O, Schmoller KM, Claessens MMAE, Bausch AR. Cytoskeletal Polymer Networks: Viscoelastic Properties are Determined by the Microscopic Interaction Potential of Cross-links. Biophysical Journal. 2009 6;96(11):4725–4732. Available from: http://www.sciencedirect.com/science/article/pii/S0006349509007589.

27. Lieleg O, Bausch AR. Cross-Linker Unbinding and Self-Similarity in Bundled Cytoskeletal Networks. Phys Rev Lett. 2007 Oct;99:158105. Available from: http://link.aps.org/doi/10.1103/PhysRevLett.99.158105.

28. Wachsstock DH, Schwarz WH, Pollard TD. Cross-linker dynamics determine the mechanical properties of actin gels. Biophysical Journal. 1994;66(3, Part 1):801 – 809. Available from: http://www.sciencedirect.com/science/article/pii/S0006349594808562.

29. Lieleg O, Claessens MMAE, Luan Y, Bausch AR. Transient Binding and Dissipation in Cross-Linked Actin Networks. Phys Rev Lett. 2008 Sep;101:108101. Available from: http://link.aps.org/doi/10.1103/PhysRevLett.101.108101.

30. Yao NY, Becker DJ, Broedersz CP, Depken M, MacKintosh FC, Pollak MR, et al. Nonlinear Viscoelasticity of Actin Transiently Cross-linked with Mutant alpha-Actinin-4. Journal of Molecular Biology. 2011;411(5):1062 – 1071. Available from: http://www.sciencedirect.com/science/article/pii/S0022283611007376.

31. Liu J, Koenderink GH, Kasza KE, MacKintosh FC, Weitz DA. Visualizing the Strain Field in Semiflexible Polymer Networks: Strain Fluctuations and Nonlinear Rheology of F-Actin Gels. Phys Rev Lett. 2007 May;98:198304. Available from: http://link.aps.org/doi/10.1103/PhysRevLett.98.198304.

32. Robin FB, McFadden WM, Yao B, Munro EM. Single-molecule analysis of cell surface dynamics in Caenorhabditis elegans embryos. Nat Meth. 2014 06;11(6):677–682. Available from: http://dx.doi.org/10.1038/nmeth.2928.

33. Fritzsche M, Lewalle A, Duke T, Kruse K, Charras G. Analysis of turnover dynamics of the submembranous actin cortex. Molecular Biology of the Cell. 2013 03;24(6):757–767. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3596247/.

34. Fritzsche M, Erlenkämper C, Moeendarbary E, Charras G, Kruse K. Actin kinetics shapes cortical network structure and mechanics. Science Advances.

2016;2(4). Available from:
`http://advances.sciencemag.org/content/2/4/e1501337`.

35. Carlsson AE. Actin Dynamics: From Nanoscale to Microscale. Annual review of
biophysics. 2010 06;39:91–110. Available from:
`http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2967719/`.

36. Lai FP, Szczodrak M, Block J, Faix J, Breitsprecher D, Mannherz HG, et al.
Arp2/3 complex interactions and actin network turnover in lamellipodia. The
EMBO Journal. 2008 04;27(7):982–992. Available from:
`http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2265112/`.

37. Van Goor D, Hyland C, Schaefer AW, Forscher P. The Role of Actin Turnover in
Retrograde Actin Network Flow in Neuronal Growth Cones. PLoS ONE.
2012;7(2):e30959. Available from:
`http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3281045/`.

38. Lenz M, Gardel ML, Dinner AR. Requirements for contractility in disordered
cytoskeletal bundles. New Journal of Physics. 2012;14(3):033037. Available from:
`http://stacks.iop.org/1367-2630/14/i=3/a=033037`.

39. Lenz M. Geometrical Origins of Contractility in Disordered Actomyosin
Networks. Phys Rev X. 2014 Oct;4:041002. Available from:
`http://link.aps.org/doi/10.1103/PhysRevX.4.041002`.

40. Murrell MP, Gardel ML. F-actin buckling coordinates contractility and severing
in a biomimetic actomyosin cortex. Proceedings of the National Academy of
Sciences. 2012;109(51):20820–20825. Available from:
`http://www.pnas.org/content/109/51/20820.abstract`.

41. Alvarado J, Sheinman M, Sharma A, MacKintosh FC, Koenderink GH. Molecular
motors robustly drive active gels to a critically connected state. Nat Phys. 2013
09;9(9):591–597. Available from: `http://dx.doi.org/10.1038/nphys2715`.

42. Murrell M, Gardel ML. Actomyosin sliding is attenuated in contractile
biomimetic cortices. Molecular Biology of the Cell. 2014;25(12):1845–1853.

Available from:
http://www.molbiolcell.org/content/25/12/1845.abstract.

43. Ennomani H, Letort G, Guérin C, Martiel JL, Cao W, Nédélec F, et al.
Architecture and Connectivity Govern Actin Network Contractility. Current
Biology. 2016;26(5):616 – 626. Available from:
http://www.sciencedirect.com/science/article/pii/S0960982216000543.

44. Reymann AC, Boujemaa-Paterski R, Martiel JL, Guérin C, Cao W, Chin HF,
et al. Actin Network Architecture Can Determine Myosin Motor Activity.
Science. 2012;336(6086):1310–1314. Available from:
http://science.sciencemag.org/content/336/6086/1310.

45. Nedelec FJ, Surrey T, Maggs AC, Leibler S. Self-organization of microtubules
and motors. Nature. 1997 09;389(6648):305–308. Available from:
http://dx.doi.org/10.1038/38532.

46. Surrey T, Nédélec F, Leibler S, Karsenti E. Physical Properties Determining
Self-Organization of Motors and Microtubules. Science. 2001;292(5519):1167–1171.
Available from: http://science.sciencemag.org/content/292/5519/1167.

47. Hiraiwa T, Salbreux G. Role of turn-over in active stress generation in a filament
network. ArXiv e-prints. 2015 Jul;.

48. Mak M, Zaman MH, Kamm RD, Kim T. Interplay of active processes modulates
tension and drives phase transition in self-renewing, motor-driven cytoskeletal
networks. Nat Commun. 2016 01;7. Available from:
http://dx.doi.org/10.1038/ncomms10323.

49. Zumdieck A, Kruse K, Bringmann H, Hyman AA, Jülicher F. Stress Generation
and Filament Turnover during Actin Ring Constriction. PLoS ONE. 2007
08;2(8):e696. Available from:
http://dx.plos.org/10.1371/journal.pone.0000696.

50. Dierkes K, Sumi A, Solon J, Salbreux G. Spontaneous Oscillations of Elastic
Contractile Materials with Turnover. Phys Rev Lett. 2014 Oct;113:148102.

Available from:
http://link.aps.org/doi/10.1103/PhysRevLett.113.148102.

51. Vanossi A, Manini N, Urbakh M, Zapperi S, Tosatti E. *Colloquium* : Modeling
    friction: From nanoscale to mesoscale. Rev Mod Phys. 2013 Apr;85:529–552.
    Available from: http://link.aps.org/doi/10.1103/RevModPhys.85.529.

52. Spruijt E, Sprakel J, Lemmers M, Stuart MAC, van der Gucht J. Relaxation
    Dynamics at Different Time Scales in Electrostatic Complexes: Time-Salt
    Superposition. Phys Rev Lett. 2010 Nov;105:208301. Available from:
    http://link.aps.org/doi/10.1103/PhysRevLett.105.208301.

53. Filippov AE, Klafter J, Urbakh M. Friction through Dynamical Formation and
    Rupture of Molecular Bonds. Phys Rev Lett. 2004 Mar;92:135503. Available
    from: http://link.aps.org/doi/10.1103/PhysRevLett.92.135503.

54. Banerjee S, Marchetti MC, Müller-Nedebock K. Motor-driven dynamics of
    cytoskeletal filaments in motility assays. Phys Rev E. 2011 Jul;84:011914.
    Available from: http://link.aps.org/doi/10.1103/PhysRevE.84.011914.

55. Chugh P, Clark AG, Smith MB, Cassani DAD, Charras G, Salbreux G, et al.
    Nanoscale Organization of the Actomyosin Cortex during the Cell Cycle.
    Biophysical Journal. 2016 2016/07/18;110(3):198a. Available from:
    http://dx.doi.org/10.1016/j.bpj.2015.11.1105.

56. Sanchez T, Chen DTN, DeCamp SJ, Heymann M, Dogic Z. Spontaneous motion
    in hierarchically assembled active matter. Nature. 2012 11;491(7424):431–434.
    Available from: http://dx.doi.org/10.1038/nature11591.

57. Broedersz CP, Storm C, MacKintosh FC. Effective-medium approach for stiff
    polymer networks with flexible cross-links. Phys Rev E. 2009 Jun;79:061914.
    Available from: http://link.aps.org/doi/10.1103/PhysRevE.79.061914.

58. Howard J, et al. Mechanics of motor proteins and the cytoskeleton. 2001;.

59. Ward A, Hilitski F, Schwenger W, Welch D, Lau AWC, Vitelli V, et al. Solid
    friction between soft filaments. Nat Mater. 2015 03;advance online publication:–.
    Available from: http://dx.doi.org/10.1038/nmat4222.

60. Chandran PL, Mofrad MRK. Averaged implicit hydrodynamic model of semiflexible filaments. Phys Rev E. 2010 Mar;81:031920. Available from: http://link.aps.org/doi/10.1103/PhysRevE.81.031920.

61. Schoenberg M. Equilibrium muscle cross-bridge behavior. Theoretical considerations. Biophys J. 1985 Sep;48(3):467–475. 4041539[pmid]. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1329360/.

62. Tawada K, Sekimoto K. Protein friction exerted by motor enzymes through a weak-binding interaction. J Theor Biol. 1991 May;150(2):193–200.

63. Leibler S, Huse DA. Porters versus rowers: a unified stochastic model of motor proteins. J Cell Biol. 1993 Jun;121(6):1357–1368.

64. Unterberger MJ, Holzapfel GA. Advances in the mechanical modeling of filamentous actin and its cross-linked networks on multiple scales. Biomechanics and Modeling in Mechanobiology. 2014;13(6):1155–1174. Available from: http://dx.doi.org/10.1007/s10237-014-0578-4.

65. Head DA, Levine AJ, MacKintosh FC. Deformation of Cross-Linked Semiflexible Polymer Networks. Phys Rev Lett. 2003 Sep;91:108102. Available from: http://link.aps.org/doi/10.1103/PhysRevLett.91.108102.

66. McCrum NG, Buckley CP, Bucknall CB. Principles of Polymer Engineering. Oxford science publications. Oxford University Press; 1997. Available from: https://books.google.com/books?id=EiqWQgAACAAJ.

67. Kim T, Gardel ML, Munro E. Determinants of Fluidlike Behavior and Effective Viscosity in Cross-Linked Actin Networks. Biophysical Journal. 2014;106(3):526 – 534. Available from: http://www.sciencedirect.com/science/article/pii/S0006349513058487.

68. Koenderink GH, Dogic Z, Nakamura F, Bendix PM, MacKintosh FC, Hartwig JH, et al. An active biopolymer network controlled by molecular motors. Proceedings of the National Academy of Sciences of the United States of America. 2009 09;106(36):15192–15197. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2741227/.

69. Gorfinkiel N, Blanchard GB. Dynamics of actomyosin contractile activity during epithelial morphogenesis. Current Opinion in Cell Biology. 2011;23(5):531 – 539. Cell-to-cell contact and extracellular matrix. Available from: `http://www.sciencedirect.com/science/article/pii/S0955067411000834`.

70. Theriot JA, Mitchison TJ. Actin microfilament dynamics in locomoting cells. Nature. 1991 Jul;352(6331):126–131. Available from: `http://dx.doi.org/10.1038/352126a0`.

71. Murthy K, Wadsworth P. Myosin-II-Dependent Localization and Dynamics of F-Actin during Cytokinesis. Current Biology. 2016 2016/12/15;15(8):724–731. Available from: `http://dx.doi.org/10.1016/j.cub.2005.02.055`.

72. Watanabe N, Mitchison TJ. Single-Molecule Speckle Analysis of Actin Filament Turnover in Lamellipodia. Science. 2002;295(5557):1083–1086. Available from: `http://science.sciencemag.org/content/295/5557/1083`.

73. Guha M, Zhou M, Wang Yl. Cortical Actin Turnover during Cytokinesis Requires Myosin II. Current Biology. 2016 2016/12/15;15(8):732–736. Available from: `http://dx.doi.org/10.1016/j.cub.2005.03.042`.

74. Fritzsche M, Lewalle A, Duke T, Kruse K, Charras G. Analysis of turnover dynamics of the submembranous actin cortex. Molecular Biology of the Cell. 2013;24(6):757–767. Available from: `http://www.molbiolcell.org/content/24/6/757.abstract`.