Kinetics of stress fibers

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Abstract. Stress fibers are contractile cytoskeletal structures, tensile acto-myosin bundles which allow sensing and production of force, provide cells with adjustable rigidity and participate in various processes such as wound healing. The stress fiber is possibly the best characterized and most accessible multiprotein cellular contractile machine. Here we develop a quantitative model of the structure and relaxation kinetics of stress fibers. The principal experimentally known features are incorporated. The fiber has a periodic sarcomeric structure similar to muscle fibers with myosin motor proteins exerting contractile force by pulling on actin filaments. In addition the fiber contains the giant spring-like protein titin. Actin is continuously renewed by exchange with the cytosol leading to a turnover time of several minutes. In order that steady state be possible, turnover must be regulated. Our model invokes simple turnover and regulation mechanisms: actin association and dissociation occur at filament ends, while actin filament overlap above a certain threshold in the myosin-containing regions augments depolymerization rates. We use the model to study stress fiber relaxation kinetics after stimulation, as observed in a recent experimental study where some fiber regions were contractile and others expansive. We find that two distinct episodes ensue after stimulation: the turnover–overlap system relaxes rapidly in seconds, followed by the slow relaxation of sarcomere lengths in minutes. For parameter values as they have been characterized experimentally, we find the long time relaxation of sarcomere length is set by the rate at which actin filaments can grow or shrink in response to the forces exerted by the elastic and contractile elements. Consequently, the stress fiber relaxation time scales...
inversely with both titin spring constant and the intrinsic actin turnover rate. The model’s predicted sarcomere velocities and contraction–expansion kinetics are in good quantitative agreement with experiment.

1. Introduction

The cytoskeleton is a highly dynamic network of semi-flexible protein filaments and other components. Two prominent elements are myosin II motor proteins and actin filaments having the important property of polarity with two distinct ends, the ‘barbed’ and the ‘pointed’ end. Since the motor domains of myosins bind to actin filaments and tend to move towards the filament barbed ends, myosins can exert contractile force when appropriately constrained. Actin structures often exhibit continuous turnover in which actin subunits exchange with cytosolic actin. Many of the cytoskeleton’s dynamical functions hinge on these two closely related properties: contractility and renewal. Cytoskeletal structures assembled from actin, myosin and other components exert contractile forces, often closely coordinated with filament assembly and disassembly. Contractile structures enable the exertion and sensing of forces, cytoskeleton remodeling, regulation of local cellular rigidity and shape and many other functions [1].

The present paper aims to elucidate mechanisms underlying the behavior of a basic cytoskeletal contractile structure: the stress fiber. We seek to understand how actin filament turnover and contractile forces may work together to generate the measured characteristics. We are particularly motivated by a recent experimental study of stress fibers where surprisingly nonuniform contractility was observed after stimulation, some fiber regions being contractile and others expansive [2].

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Stress fibers are tensile actomyosin bundles anchored at each end to the extracellular matrix via transmembrane protein complexes called focal adhesions. They are both force producers and force sensors [3, 4] and can endow the cell with anisotropic dynamically adjustable rigidity as required [5]. In vivo, stress fibers arise in vascular endothelial cells under shear stress [6, 7] where they mediate adhesion to the underlying extracellular matrix (the basal lamina) and in contractile cells (myofibroblasts) involved in wound healing [8] which exert forces to close the wound [9]. Beyond its intrinsic importance, the stress fiber is possibly the simplest and most accessible cellular actomyosin contractile machine and establishing its organization and mechanisms may illuminate general cortical contractility and presumably more complex structures such as the contractile ring in cytokinesis [10, 11].

Along the stress fiber axis regions containing the actin cross-linking protein α-actinin alternate with regions containing myosin [12] and the polarity of the actin is periodic [13]. In addition, a nonmuscle form of the giant spring-like protein titin, c-titin, localizes to stress fibers in a periodic pattern [14]. These observations are consistent with a sarcomeric structure similar to that of muscle fibrils [15, 16], albeit one that may be somewhat less ordered. This is shown schematically in figure 1: the myosin-containing regions (analogous to the A-bands in muscle) lie in the middle of each sarcomere which is symmetric about its center plane; two oppositely-oriented actin filaments of equal length lie on either side of the center plane, their pointed ends in the myosin region and their barbed ends near the sarcomere boundaries in the α-actinin-containing regions (analogous to the Z-line regions in muscle). One sarcomere consists of many such filament pairs plus myosin arranged in parallel into an approximately cylindrical bundle, while the stress fiber is built from many such sarcomeres connected in series.

The contractile force in stress fibers is produced by nonmuscle myosin II molecular motors, likely in the form of bipolar minifilaments [17, 18], aggregates of 10–30 myosin molecules approximately 0.3 µm in length [19]. Similar to thick filaments in striated muscle, minifilaments are symmetric with myosin motor domains located at both ends and separated by a bare zone. Thus, similarly to muscle, according to the scheme of figure 1 minifilaments exert inward contractile force since myosins move towards actin barbed ends. Motor activity is regulated biochemically by phosphorylation of the myosin regulatory light chain [20]: when phosphorylated (unphosphorylated) a given myosin molecule is active (inactive). Each sarcomere has multiple minifilaments which may be aggregated as observed previously in fibroblast lamellipodia [19].

Because stress fibers in stationary cells usually exert isometric tension and changes are slow, it has been difficult to observe their dynamics and hence identify mechanisms. However, a recent experimental study has provided the first measurements of the kinetics of intact stress fibers in living cells. Using agents that increase myosin phosphorylation, Peterson et al [2] stimulated the myosin in stress fibers of living fibroblasts. Tagging myosin II regulatory light chain and α-actinin with green fluorescent protein, time-dependent sarcomere lengths were tracked, providing detailed kinetic information previously unavailable. Interestingly, the effect of stimulation was dramatically spatially nonuniform: myosin in the peripheral regions became more phosphorylated than myosin in the center and then, over the course of ~10 min, peripheral sarcomeres contracted while those close to the fiber center expanded. While the overall length of the fiber decreased little, individual sarcomeres changed lengths considerably. Under normal conditions all sarcomeres were approximately 1 µm in length, while sarcomeres contracted or expanded by as much as 0.5 µm after stimulation.
In the present paper, we develop a quantitative model of stress fibers based on current knowledge, and we apply our model to describe the experiments of Peterson et al. Previous theoretical work on actomyosin fibers [21, 22] addressed nonpolar bundles motivated in part by experiments in which disordered bundles of actin and myosin fragments in the absence of passive crosslinkers contracted in vitro [23]. These bundles are very different from stress fibers, which have a more ordered structure, passive crosslinkers, and actin turnover (discussed below). The formation and alignment of many stress fibers in a cell has been theoretically studied [24]–[26], but these works addressed collective behavior and did not attempt detailed descriptions of individual stress fibers. Denoth et al [27] modeled muscle fibrils and studied how sarcomeres having different properties influence each other, which is related to our interest here. However, no model exists which relates stress fiber mechanisms to physical structure.

In addition to myosin’s active contractile force, our model includes a passive elastic force due to cellular titin. The recent discovery that c-titin is associated with stress fibers in a periodic spatial pattern strongly suggests the role of a template, similar to that in muscle, which maintains

Figure 1. Schematic of the stress fiber model developed in the present paper. (a) Along the stress fiber axis, regions containing myosin (blue) alternate with regions containing α-actinin (green). Each end of the fiber connects to a focal adhesion, a transmembrane protein complex that is anchored to the extracellular matrix. (b) Considerable evidence indicates stress fibers have a sarcomeric structure built from actin (gray), myosin (blue), and titin (orange). Our model incorporates a contractile force $f_{\text{myo}}$ from the motor activity of nonmuscle myosin II minifilaments depending on the relative myosin-actin velocity $v_{\text{myo}}$ and a passive elastic force $f_{\text{titin}}$, proportional to sarcomere length $x$, from the giant protein c-titin. The fiber tension $T$ is transmitted from neighboring sarcomeres and is expansive. Also acting to expand the sarcomere is the force $p$ resisting overlap of actin filaments at their pointed ends (shown schematically as if resulting from bending of overlapped filaments). The overlap force is a function of the amount of overlap $z$ and tends to augment the pointed end depolymerization rate $v_-$. Actin polymerization occurs at constant rate $v_+$ at barbed ends. The barbed ends lie in the α-actinin-containing regions (shown green in part (a)) somewhat analogous to the Z-lines in striated muscle. Note $v_+$ and $v_-$ are defined per sarcomere so each is the sum of identical contributions from the two oppositely oriented actins defining the sarcomere.
the periodic α-actinin–myosin arrangement [14] and provides passive elasticity in response to sarcomere length changes tending to undo the templated arrangement. Single molecules of titin from muscle cells have elastic constants $k_t \approx 3.75 \text{pN} \mu\text{m}^{-1}$ at lengths relevant for stress fibers [28]. In fact, we find that stress fibers cannot reach steady state without the inclusion of such passive elasticity; within our model’s general framework, we are forced to introduce a titin-like presence to explain the experiments of [2].

The final element in our model is actin turnover, which plays a central role. Several experimental studies have shown that actin in stress fibers turns over on a timescale of minutes [4, 29, 30], similar to the sarcomere expansion/contraction timescales seen in [2] and suggesting a possible role in the kinetics. Thus actin polymerization/depolymerization processes occur along the fibers, possibly localized to specific centers of activity. Actin has been observed to preferentially incorporate into the Z-line-like α-actinin regions [31], where the barbed ends are located. This suggests growth at the barbed ends, possibly assisted and/or regulated by protein machinery. It is natural to speculate that formins may assume such a role [32], since they are known to nucleate and regulate growth of unbranched actin filaments of the type stress fibers contain. However, while formins are involved in the creation of stress fibers [30, 33], no evidence exists to date for their presence in mature fibers. Turning to depolymerization, this may occur at the relatively unstable pointed ends. Another possibility is that the actin severing protein coflin is involved; stress fibers thicken and become more prominent in coflin knockdown cells [34].

Given the poor current understanding of turnover, we adopt the simplest possible model, assuming growth at the barbed ends at rate $v_+$ (filament length per second), and depolymerization rate $v_-$ at the pointed ends. A key point is that in steady state these are equal, $v_+ = v_-$, and hence one or both must presumably be regulated: when a filament becomes too long or short, polymerization and/or depolymerization rates will adjust accordingly. That is, filament length and polymerization/depolymerization rates are presumably coupled. (A proposed mechanism whereby $v_+$ and $v_-$ adjust independently of filament length suffers from the difficulty that even when $v_+ = v_-$ growth rate fluctuations, characterized by a ‘length diffusivity’, would lead to uncontrolled growth or attrition [35].) This coupling may be mechanical or biochemical. Here for simplicity we take $v_+$ fixed and allow $v_-$ to be regulated via the following mechanical mechanism. For a given sarcomere length, the longer the filaments the greater the overlap of the oppositely oriented filaments at their pointed ends in the A-band-like myosin regions; this overlap will eventually build up a resistance force, if only because only so much actin filament can fit into the limited space and specific geometry. This force is the feedback, increasing $v_-$ and allowing a steady state overlap where $v_+ = v_-$. The two essential ingredients are: (i) resistance force dependence on overlap and (ii) depolymerization rate dependence on resistance force. Our picture is motivated in part by the observation that the pointed ends of actin in stress fiber sarcomeres almost always overlap [36] and that overlap forces arise in striated muscle, often described with a length-dependent tension [37].

A key parameter in our model is $p^*$, the characteristic overlap force which must be attained before depolymerization is significantly increased. Though we invoke a specific feedback mechanism here, we propose that regardless of the details of the actual depolymerization-length coupling mechanism certain basic features identified by this model have generality. An example is $p^*$, the characteristic force where polymerization/depolymerization rates become modified.
What forces does turnover generate? This is simplest to see in steady state, where actin filaments treadmill at rate \( v_+ \). It follows that the myosins are effectively walking along their actin tracks with velocity \( v_+ \) (see figure 1) and the force they exert is reduced from the stall force by \( \lambda v_+ \) where \(-\lambda\) is the slope of the myosin force–velocity relation near stall (assuming a small force reduction). Equivalently, one can think of myosins as always pulling with the stall force and \( \lambda \) as an effective internal drag coefficient provided by the myosin system; \( \lambda v_+ \) is then the viscous drag force due to treadmilling, which will contribute to the stress fiber tension. This illustrates how the tension in general depends on turnover rates.

We will see that the magnitude of the turnover rate is naturally measured by an important parameter,

\[
  r \equiv \lambda v_+ / p^*. 
\]

For the experiments of [2], we estimate \( r \approx 0.1 \). In this slow turnover limit, \( r \ll 1 \), the dissipative turnover force is small but we find that turnover governs the sarcomere relaxation kinetics: one of our principal predictions is that the sarcomere relaxation time is \( \tau_{\text{sarc}} = p^*/(k_v v_+) \). Applied to the system of [2] this yields a relaxation time of order minutes, in agreement with the observed kinetics.

The plan of the present paper is as follows. In section 2, we detail the various components of a stress fiber and assemble our model equations. In section 3, isotonic boundary conditions are considered, the simplest case where sarcomeres are independent which nonetheless reveals most of the physics of stress fiber kinetics. We find that the overlap force and depolymerization rate are fast variables, relaxing very rapidly to a quasi-steady state and subsequently following the slow sarcomere length variables. Two possible limiting cases emerge, large turnover rate \( (r \gg 1) \) and small turnover rate \( (r \ll 1) \), exhibiting qualitatively different behavior. We argue that typical stress fiber systems, in particular those of [2], belong to the \( r \ll 1 \) case. Isometric boundary conditions are treated in section 4, in which coupling between sarcomeres is necessary to enforce constant length. In section 5, we apply our model to the experiments of Peterson et al [2] and demonstrate that the predicted dynamics are in good agreement with the experiment. We conclude with a discussion.

### 2. The model

Each end of the stress fiber of \( N \) sarcomeres, \( n = 1, 2, \ldots, N \), is connected to a focal adhesion, a transmembrane protein complex adhered to the extracellular matrix. Figure 1 shows a schematic of the structure of one sarcomere and the forces involved. The total contractile force exerted by a myosin minifilament is

\[
  f_{\text{myo}} = f_s - \lambda v_{\text{myo}},
\]

where \( f_s \) is the stall force per minifilament and \( v_{\text{myo}}/2 \) the myosin velocity relative to its actin track. Thus \( v_{\text{myo}} \) is defined for convenience to be the sum of the myosin working speeds at each end of the minifilament. \(-\lambda\) is the slope of the myosin force–velocity relation at the stall force. Figure 2 shows schematically such a relation with a form similar to that measured for muscle [15]. To our knowledge, no such measurements exist for nonmuscle myosin minifilaments. For our main purpose here, analysis of the experiments of [2], (2) is an accurate description since the observed velocities were very small, \( v_{\text{myo}}/2 \ll v_{\text{myo}}^0 \), where \( v_{\text{myo}}^0 \) is the zero-load myosin working velocity (see figure 2). The only relevant feature of the force-velocity relation is the magnitude of \( \lambda \).
Figure 2. Proposed form of the force-velocity relation for a nonmuscle myosin minifilament. The net force generated by the myosins, $f_{\text{myo}}$, is shown as a function of twice the actin filament–minifilament relative velocity, $v_{\text{myo}}$. (Thus $v_{\text{myo}}$ would be the sarcomere shrinkage rate in the absence of polymerization.) Principal features include the zero-load myosin working velocity, $v_0^{\text{myo}}$, the stall force $f_s$ and the slope at the stall force, $-\lambda$, which can be interpreted as a myosin internal drag coefficient. Only $v_0^{\text{myo}}$ has been measured directly for nonmuscle myosin II, found to be $v_0^{\text{myo}} \approx 300$ nm s$^{-1}$ in gliding assays [45, 46]. We assume that the stall force per myosin molecule and the shape of the force-velocity relation are as for muscle myosin II. The internal myosin drag coefficient is $\lambda = \beta f_s/(2v_0^{\text{myo}})$ where $f_s/(2v_0^{\text{myo}})$ would be the coefficient for a linear force-velocity relation and $\beta$ accounts for curvature. We set $\beta = 5$, the value for striated muscle [15, 43].

Stress fibers contain a form of titin arranged periodically along the fiber axis [14]. Its sequence is very similar to that of striated muscle titin [14], which passively resists stretching of the sarcomere [38]. Cellular titin is likely to have a similar role in stress fibers, so we assume an elastic restoring force. Assuming similar properties to muscle titin [28], for the range of experimentally observed sarcomere lengths ($\sim 1 \mu$m) a relation linear in sarcomere length $x$ is valid:

$$f_{\text{titin}} = k_t x,$$

where $k_t$ is the titin force constant whose value in muscle is $k_t \approx 3.75$ pN $\mu$m$^{-1}$ [28]. In vitro, titin assembles with myosin into stress-fiber-like structures in a one-to-one ratio [39]. Thus we assume one titin molecule per minifilament, and (3) represents the force per minifilament.

Evidence suggests that opposing actin filaments in stress fibers are normally overlapped at the pointed ends in the central A-band-like zone [36], unlike striated muscle where there is no overlap over much of the range of operating sarcomere lengths [40]. Here we assume overlap above a certain threshold generates a resistance force $p$ per minifilament which for simplicity
follows a linear law,
\[ p = k z, \quad (z > 0), \]
where \( k \) is the force constant and \( z \) is the amount of overlap above the threshold value. The force vanishes for \( z < 0 \). Its precise origin is unknown but contributions may include simple excluded volume, among opposing actin filaments or actin filaments and myosin, and actin filament bending.

As discussed in the introduction we account for actin turnover by assuming simple treadmilling of actin monomers, adding at constant rate \( v_+ \) at the barbed ends and dissociating at regulatable rate \( v_- \) at the pointed ends (see figure 1). Note that these rates are defined per sarcomere, and thus are the sum of identical contributions from actin filaments of both polarities. At one or both ends cappers or other polymerization machinery may be involved, but this is unknown. Since the pointed end overlap force \( p \) is caused by filament growth, it is assumed to influence pointed end depolymerization according to
\[ v_- = v_0^0 \exp^{p/p^*}, \]
where \( p^* \) is a characteristic force level at which this feedback becomes substantial. Here \( v_0^0 \) is the force-free depolymerization velocity. From general considerations one expects a linear dependence at small \( p \) and a strong possibly exponential form at large \( p \) \cite{41}. The particular form of (5) is simply a convenient interpolation between these two limits and does not qualitatively affect our results. The only important feature is that depolymerization is amplified at some characteristic force, \( p^* \).

Let us now assemble the sarcomere dynamics from the above forces according to the scheme of figure 1. Consider a given sarcomere of length \( x \) in a fiber at tension \( T \) per myosin minifilament. The force balance on this sarcomere reads
\[ T = f_{\text{myo}} + f_{\text{titin}} - p, \]
and the myosin working velocity is
\[ v_{\text{myo}} = v_+ - \dot{x}, \]
where the dot denotes differentiation with respect to time.

Thus, combining (2)–(4), (6) and (7), the length kinetics are
\[ \lambda \ddot{x} = -k_t x + k z - f_s + \lambda v_+ + T, \]
\[ \dot{x} + \dot{z} = v_+ - v_0^0 \exp^{k z/p^*}. \]
Equations (8) and (9), plus appropriate boundary conditions (for example, isotonic or isometric) and initial conditions on length \( x \) and overlap \( z \) are a closed system.

In the following, we have in mind experiments where initially a fiber is far from steady state. In \cite{2} this is induced by a position-dependent phosphorylation stimulus, such that stall force \( f_s \) and internal drag coefficient \( \lambda \) are different in different sarcomeres. Thus \( x(0) \) and \( z(0) \) deviate from their \( t \to \infty \) values for the given sarcomere. It is understood that \( x, z, f_s \) and \( \lambda \) are in general different in different sarcomeres.

Steady state values will be denoted by subscript \( \infty \). We will often deal with the deviations from steady state,
\[ y \equiv x - x_\infty, \quad \delta z \equiv z - z_\infty, \quad \delta T \equiv T - T_\infty. \]
3. Stress fiber at constant tension

Under isotonic conditions the stress fiber is held at constant tension while the total length, \( L = \sum_{n=1}^{N} x_n \), can vary. Thus sarcomeres are independent of each other, a considerable simplification. This will help us to understand the more complex and probably more physiologically important isometric kinetics (section 4) which are realized in the experiments of [2]. Moreover, an intriguing possibility would be to create isotonic conditions in experiments involving controlled deformable substrates.

In what follows, we assume that at \( t = 0 \) the fiber is perturbed from steady state, for example, by a sudden change in applied tension or myosin phosphorylation profile. First, let us establish what the steady state will be under these new conditions. Then we analyze the process of relaxation to this new steady state.

3.1. Steady state

In steady state, the polymerization and depolymerization rates are equal. This can only be achieved by the overlap \( \tilde{z} \) adjusting to the value \( \tilde{z}_\infty \) such that \( v_- = v_+ \). Thus, from (9),

\[
\tilde{z}_\infty = z^* \ln \left( \frac{v_+}{v_-^0} \right), \quad z^* = p^*/k.
\]  

(11)

\( z^* \) is the characteristic overlap where depolymerization is substantially promoted. Clearly, the condition \( v_+ > v_-^0 \) must be satisfied for this mechanism of turnover regulation. Since depolymerization responds with exponential strength, even away from steady state the overlap \( \tilde{z} \) will normally be of order \( z^* \) or less. From (8), the steady state sarcomere length \( x_\infty \) is given by

\[
k_t x_\infty = T - f_s + \lambda v_+ + k\tilde{z}_\infty.
\]  

(12)

Note the effect of turnover: both the polymerization term, \( \lambda v_+ \), and that associated with depolymerization, \( k\tilde{z}_\infty \), tend to lengthen the sarcomere for a given tension. The former is the dissipative work done by myosin against internal viscosity to maintain the steady state treadmilling rate \( v_+ \); the latter is the compressive overlap force needed to equalize the actin on and off rates.

3.2. Relaxation kinetics: two timescales

A striking and simplifying feature of the kinetics now emerges: it turns out that the overlap \( z \) is a fast variable, enslaved to the slow sarcomere length variable \( x \). Our procedure will be to first assume the two relevant timescales separate, and then after solving the dynamics perform a self-consistency check which yields a necessary condition for this to be true; we will see that for realistic parameter values this condition is indeed satisfied. Typically, overlap and polymerization rate relax in order seconds, while length relaxation requires minutes.

It is convenient to deal with the sarcomere length and overlap relative to their ultimate steady state values, \( y \) and \( \delta z \). Strong sarcomeres (\( y > 0 \)) will contract, whereas weak sarcomeres (\( y < 0 \)) will expand. In this language (8) and (9) become

\[
\lambda \dot{y} = -k_t y + k \delta z,
\]  

(13)

and

\[
\dot{y} + \dot{z} = v_+ - v_-^0 e^{\delta z^*/z^*}.
\]  

(14)
Eliminating $\dot{y}$ from (13) and (14) yields the overlap dynamics:

$$\lambda \dot{z} = \lambda v_+ (1 - e^{\delta z / z^*}) - k \delta z + k_1 y,$$

(15)

after using (11). Near steady state the overlap $z$ is of order $z^*$, so the two $z$-dependent terms have characteristic values $\lambda v_+$ and $p^*$, respectively. The dimensionless turnover parameter $r \equiv \lambda v_+ / p^*$, introduced in (1), measures the relative magnitude of this pair. This is a key parameter: the physics of relaxation are qualitatively different in the two cases of small and large $r$, respectively.

3.3. Fast turnover, $r \gg 1$

In this case the myosin-associated drag force is much larger than the overlap force, so the overlap dynamics (15) simplify to

$$\lambda \dot{z} \approx \lambda v_+ (1 - e^{\delta z / z^*}) + k_1 y.$$

(16)

Assume $y$ is slowly varying and can be treated as approximately constant in (16). Then in a timescale $\sim \tau_{olap}$ the overlap relaxes to a value obeying

$$\lambda v_+ (1 - e^{\delta z / z^*}) \approx -k_1 y, \quad \tau_{olap} = z^* / v_+.$$

(17)

This is the quasi-steady state relation, rapidly established in time $\sim \tau_{olap}$. It describes the subsequent enslavement of the overlap variable $z$ to the slow sarcomere length variable $y$.

To better understand the nature of this early relaxation process, note that for large enough $r$ we can expand the exponential (16) and (17) so the short time dynamics and quasi-steady state approximate

$$\dot{z} \approx -\frac{v_+ \delta z}{z^*} + \frac{k_1 y}{\lambda}, \quad k \delta z = k_1 y / r \quad (t > \tau_{olap}).$$

(18)

Provided $r > k_1 y / p^*$ expanding the exponentials is valid. It follows that the $k \delta z$ term can be neglected in (13) and hence the relative change in $y$ by the time $\tau_{olap}$ is order $k_1 / (r k) \ll 1$. Thus during the fast phase sarcomere length is essentially constant, $y = y_0 \equiv y(t = 0)$, as is the sarcomere velocity $\dot{y} \approx -k_1 y_0 / \lambda$. Noting that the rate of increase in the filament length $l_{fil}$ is equal to $v_+ - v_-$, the overlap dynamics (18) can be rewritten as $\dot{z} = dl_{fil} / dt - \dot{y}$. This articulates the physical process during the fast phase as follows. Considering a contractile sarcomere as an example, initially the overlap increases as the sarcomere shrinks at constant filament length, $\dot{z} = -\dot{y}$ (since initially $dl_{fil} / dt = 0$); but this increase in overlap increases the depolymerization rate, so now $dl_{fil} / dt < 0$. This continues until in quasi-steady state the rate of decrease of filament length just matches the constant sarcomere shrinking rate. Since $\dot{y}$ is a constant, the fast timescale for $\dot{z}$ to relax is the polymerization relaxation time in (18), $z^* / v_+$. When quasi-steady state is reached the overlap is fixed (or more precisely, it is changing very slowly) and the filament length is decreasing as it ‘melts’ into the overlap region. Overall, during the early fast phase overlap is tuned until the filament grows or shrinks together with, and at the same rate as, the shrinking or growing sarcomere.

For longer times, $t \gg \tau_{olap}$, sarcomere lengths change slowly with overlap enslaved according to (18), $k \delta z = k_1 y / r$. Note the deviation of the overlap from its steady state value is very small. The overlap and hence filament shrinkage rate decrease in proportion to the sarcomere excess length $y$. The slow $y$ dynamics are obtained by using the quasi-steady state relationship for $z$, (17), in (14):

$$\lambda \dot{y} \approx -k_1 y, \quad y = y_0 e^{-t / \tau_{sarc}}, \quad \tau_{sarc} = \lambda / k_1,$$

(19)
where \( y_0 \) is the initial value of \( y \). Note that for these times the overlap is relaxed and the \( \dot{z} \) term negligible. Thus, sarcomere length has simple exponential relaxation kinetics. The relaxation time \( \tau_{\text{sarc}} \) is identified as the time required for the sarcomere to change length so the force imbalance caused by the initial perturbation is corrected by a suitable stretch or contraction of titin, the titin forces working against the myosin-derived internal drag coefficient \( \lambda \). The timescale does not feature \( v_* \), reflecting the fact that the filament length is continuously and very rapidly readjusted to the current conditions and actin growth/shrinkage is not rate limiting. Interestingly, under all conditions in this model, the presence of titin is required for the fiber to reach a new steady state.

The self-consistency condition for timescale separation is

\[
\frac{\tau_{\text{olap}}}{\tau_{\text{sarc}}} = \frac{k_t}{(kr)} \ll 1. \tag{20}
\]

Now we will see later that the overlap spring constant \( k \) typically exceeds that of the weak titin spring, \( k_t \approx 3.75 \text{ pN \mu m}^{-1} \) [28]. Thus the timescales are well separated and the above procedure is validated for large \( r \).

### 3.4. Slow turnover, \( r \ll 1 \)

We will argue in section 5 that for real stress fibers the turnover parameter \( r \) is normally very small. Then the overlap resistance is much larger than the internal drag term in the short time \( z \) dynamics (15), which now approximate

\[
\lambda \dot{z} \approx -k \delta z + k_t y. \tag{21}
\]

Treating the slow \( y \) variable as constant, it follows that the overlap relaxes (\( \delta z \sim 1 - e^{-t/\tau_{\text{olap}}} \)) after time \( \tau_{\text{olap}} \) to a quasi-steady state value obeying

\[
k \delta z = k_t y, \quad \tau_{\text{olap}} = \frac{\lambda}{k}. \tag{22}
\]

Whereas for large \( r \) the overlap relaxation time derived from turnover, in the present case of small turnover the filament length is approximately fixed and the quasi force balance, (22), is achieved by translating the entire filament by adjusting the overlap. Since \( y \) is assumed to change little in \( \tau_{\text{olap}} \), only the overlap force \( p \) changes substantially during this process. It thus sets the timescale, \( \tau_{\text{olap}} = \frac{\lambda}{k} \), working against the weak myosin internal drag. For instance, if the tension was reduced at \( t = 0 \), the overlap force would quickly increase to balance the now effectively stronger myosin. From (14) with the small turnover terms discarded, the changes during \( \tau_{\text{olap}} \) obey \( \Delta y \approx -\Delta z \). Thus in order that the relative change in \( y \) be small, it is required that \( k \gg k_t \) be satisfied. This condition is expected to be satisfied for actual stress fibers (see section 5).

Using this quasi-steady state \( z \) value in (14) with \( \dot{z} \) neglected, the slow \( y \) dynamics are

\[
\dot{y} = v_*(1 - e^{k_t y/p^*}). \tag{23}
\]

Thus,

\[
y = -(p^*/k_t) \ln[1 + (e^{-y_0 k_t/p^*} - 1)e^{-t/\tau_{\text{sarc}}}], \quad \tau_{\text{sarc}} = p^*/(k_t v_*). \tag{24}
\]

If the initial value \( y_0 \) exceeds \( p^*/k_t \), then equation (24) describes an initial rapid phase where \( y \) drops from \( y_0 \) to \( \sim p^*/k_t \), followed by the late behavior

\[
y \approx (p^*/k_t)e^{-t/\tau_{\text{sarc}}}, \quad t \gg \tau_{\text{sarc}}. \tag{25}
\]
During this relaxation, the titin spring force must adjust for the sarcomere length to reach a new steady state. Since the overlap changes very little during quasi-steady state \((\Delta z/\Delta y = k_t/k \ll 1)\) from (22), the actin filaments must change length if the sarcomere is to change length. This length change depends on the slow turnover processes which are rate limiting. Hence the turnover ‘viscous drag’, \(p^*/v_s\), sets the relaxation time \(\tau_{\text{sarc}}\).

The condition for timescale separation is
\[
\frac{\tau_{\text{olap}}}{\tau_{\text{sarc}}} = \left(\frac{k_t}{k} \right)r \ll 1,
\]
which is always satisfied for this slow turnover case.

3.5. Connection between the \(r\) parameter and timescales

It is illuminating to note that the turnover parameter \(r\) can be written as the ratio of the overlap relaxation timescales in the two cases,
\[
r = \frac{\lambda}{z^*/v_s}.
\]
These timescales originate in the two different mechanisms for changing the overlap: turnover and overlap force. The fastest process sets the overlap relaxation time.

Similarly, \(r\) can be written as the ratio of the two sarcomere relaxation timescales:
\[
r = \frac{\lambda}{p^*/(k_t v_s)}.
\]
In contrast to the short time overlap dynamics, the slowest mechanism sets the timescale for long time sarcomere relaxation.

4. Stress fiber at constant length

Under isometric conditions the total length of the stress fiber, \(L = \sum_{n=1}^{N} x_n\), is constant. This is probably the more physiologically relevant condition because stress fiber ends are often connected to immobile focal adhesions. Our particular interest in the present paper is to model the experiments of Peterson et al [2] (see section 5) where conditions were approximately isometric: though focal adhesions slowly moved centripetally as the stress fiber contracted, the relative change in the entire fiber length was significantly less than the relative length changes of individual sarcomeres.

We consider an initially uniform fiber, every sarcomere having length \(l = L/N\). Note the mean length will equal \(l\) at all times. At \(t = 0\), a perturbation is imposed such that the myosin activity profile is nonuniform along the fiber. We assume the myosin heads of a sarcomere work independently in parallel, so changing the number of active heads changes the maximum force that can be generated but not the maximum velocity. Thus the slope \(\lambda\) increases in proportion to the stall force \(f_s\). The activity of a given sarcomere is characterized by the relative deviation of its stall force and slope from the mean values:
\[
\epsilon \equiv \frac{f_s - \bar{f}_s}{\bar{f}_s} = \frac{\lambda - \bar{\lambda}}{\bar{\lambda}}.
\]
Angular brackets and overbar will be used interchangeably to denote averages over all sarcomeres, e.g. \(\bar{\lambda} \equiv \langle \lambda \rangle\). A sarcomere with \(\epsilon > 0\) (\(\epsilon < 0\)) is relatively strong (weak). Average values such as \(\langle \epsilon^2 \rangle\) represent the variation in sarcomere activities across the entire fiber.
In the experiments of [2] the variation in measured phosphorylation levels was small, \(|\epsilon| \ll 1\). In this case one can obtain explicit analytical expressions which clearly articulate the basic mechanisms. We specialize to this case at the end of this section.

4.1. Steady state

In steady state the overlap must adjust to equalize the actin on and off rates, so \(z_\infty\) is unchanged from the expression for the isotonic case, (11). Averaging (8) in steady state over all sarcomeres gives the steady state tension,

\[
T_\infty = \bar{f}_s - v_+ \bar{\lambda} + k_t l - k z_\infty. \tag{30}
\]

Note that if a perturbation changes the average level of myosin activation then the steady state tension will change by an amount \(\Delta T_\infty = \Delta \bar{f}_s - v_+ \Delta \bar{\lambda}\).

Subtracting (8) from its average, both at steady state, gives the steady state sarcomere length

\[
k_t x_\infty = k_t l - \epsilon \bar{f}_s + v_+ + \epsilon \bar{\lambda}. \tag{31}
\]

Stronger sarcomeres \((\epsilon > 0)\) will end up shorter than weaker ones \((\epsilon < 0)\).

4.2. Relaxation kinetics

In terms of the relative variables, the sarcomere dynamics (8) and (9) are

\[
\lambda \dot{y} = -k_t y + k \delta z + \delta T(t), \quad \dot{y} + \dot{z} = v_+ (1 - e^{\delta z/z^*}). \tag{32}
\]

These are as for isotonic conditions except for the new time-dependent tension term. Just before the \(t = 0\) perturbation the fiber was uniform and at steady state, so using (31) the initial conditions \((t = 0^+)\) are

\[
k_t y_0 = \epsilon (\bar{f}_s - v_+ \bar{\lambda}), \quad z(0) = z_\infty. \tag{33}
\]

The tension is determined by the isometric constraint. Dividing the first expression of (32) by \(\lambda\) and averaging,

\[
\delta T = \frac{k_t \langle y/\lambda \rangle - k \langle \delta z/\lambda \rangle}{\langle 1/\lambda \rangle}. \tag{34}
\]

It follows that the initial tension is lower than \(T_\infty\)

\[
\delta T(0) = \frac{(\epsilon/(1 + \epsilon))}{(1/(1 + \epsilon))} (\bar{f}_s - v_+ \bar{\lambda})
\approx - (\epsilon^2) (\bar{f}_s - v_+ \bar{\lambda}), \quad (|\epsilon| \ll 1), \tag{35}
\]

after using \(\lambda = (1 + \epsilon)\bar{\lambda}\). Interestingly, for small \(\epsilon\) this deviation is second order, proportional to the squared sarcomere strength fluctuation.

Eliminating \(\dot{y}\) from (32) gives the isometric overlap dynamics

\[
\lambda \dot{z} = \lambda v_+ (1 - e^{\delta z/z^*}) - k \delta z + k_t y - \delta T. \tag{36}
\]
4.3. Fast turnover, \( r \gg 1 \)

Discarding the overlap force term in (36), the overlap relaxation kinetics are
\[
\lambda \dot{z} \approx \lambda v_+ (1 - e^{\delta z/z^*}) + k_1 y - \delta T. \tag{37}
\]

The overlap relaxes after time \( \tau_{\text{olap}} \) to the following quasi-steady state relation
\[
\lambda v_+ (1 - e^{\delta z/z^*}) \approx -k_1 y + \delta T, \quad \frac{\delta z}{z^*} = \ln \left[ 1 + \frac{k_1 y - \delta T}{\lambda v_+} \right], \quad t \gtrsim \tau_{\text{olap}} = \frac{z^*}{v_+}. \tag{38}
\]

Thus the long time slow dynamics (32) are
\[
\lambda (\dot{y} + \dot{z}) \approx -k_1 y + \delta T. \tag{39}
\]

Now from (38)
\[
\dot{z} = \left( \frac{k_1 \dot{y}}{kr} - \frac{\delta T}{kr} \right) \ln \left[ 1 + \frac{k_1 y - \delta T}{\lambda v_+} \right]. \tag{40}
\]

Inserting this into (39), the first term \( \sim k_1 \dot{y} / (kr) \) is negligible while the second term \( \sim \delta T / (kr) \) relaxes to essentially zero after the short timescale \( \tau_{\text{olap}} \). It follows that \( \dot{z} \) can be discarded in the slow dynamics:
\[
\dot{y} + \frac{k_1 y}{\lambda} \approx \frac{\delta T}{\lambda}, \quad \delta T = \frac{k_1 (y/\lambda)}{(1/\lambda)}, \tag{41}
\]
where the slow tension dynamics were obtained by averaging the sarcomere length dynamics whose relaxation time is of order
\[
\tau_{\text{sarc}} = \tilde{\lambda}/k_1. \tag{42}
\]

Note that the ratio of timescales is very small, \( \tau_{\text{olap}} / \tau_{\text{sarc}} \approx k_1 / (kr) \), justifying the separation of the fast and slow dynamical episodes when the polymerization parameter \( r \) is large.

Solving the length dynamics (41) analytically is difficult in general. However, when the variation among sarcomeres is small, \( \epsilon \ll 1 \), the leading order solution is easily obtained as follows. Using \( \lambda = (1 + \epsilon) \tilde{\lambda} \) we have
\[
\delta T = -\langle \epsilon k_1 y \rangle, \quad \dot{y} + \frac{k_1 y}{\lambda} = 0, \quad (\epsilon \ll 1), \tag{43}
\]

having solution
\[
k_1 y(t) = \epsilon (\tilde{f}_s - v_+ \tilde{\lambda}) e^{-t/\tau_{\text{sarc}}}, \quad \delta T = -\langle \epsilon^2 \rangle (\tilde{f}_s - v_+ \tilde{\lambda}) e^{-t/\tau_{\text{sarc}}}, \tag{44}
\]
where we used the initial condition (33) and the fact that \( y \) changes very little during the fast episode. Thus while sarcomere length deviations are first order, tension deviations are very small and second order, proportional to the squared fluctuation in phosphorylation levels across the fiber.

4.4. Slow turnover, \( r \ll 1 \)

Discarding the turnover term in (36), the overlap dynamics approximate
\[
\lambda \dot{z} \approx -k \delta z + k_1 y - \delta T, \quad \delta T \approx \frac{k_1 (y_0 / \lambda) - k \langle \delta z / \lambda \rangle}{\langle 1/\lambda \rangle}. \tag{45}
\]

The expression for the tension was obtained as follows. In this small polymerization limit the filament length is approximately unchanged on the overlap relaxation timescale \( \tau_{\text{olap}} \).
i.e. the turnover terms in (32) effectively vanish whence \( \langle \dot{z} \rangle = -\langle \dot{y} \rangle = 0 \). Thus the mean relative overlap \( \langle \delta z \rangle \), initially zero, remains approximately zero throughout this early episode. Dividing the overlap dynamics in (45) by \( \lambda \) and averaging then gives the expression for \( \delta T \) after assuming \( y \) changes negligibly during \( \tau_{\text{olap}} \). Note that the same result is available directly from (34).

In solving the fast dynamics system (45) the length variable \( y \) is treated as constant; self-consistently, therefore, its relative change must be small. This is a closed system for the \( N \) overlaps and the tension, having \( N \) eigenvalues of order \( \hat{\lambda} / k \). Thus after a timescale of order \( \tau_{\text{olap}} \) it will relax to the quasi-steady state relation

\[
k \delta z = k_y y - \delta T, \quad (t > \tau_{\text{olap}} = \hat{\lambda} / k).
\]

Our starting point for the slow \( y \) dynamics is the length constraint (32)

\[
\dot{y} = v_e (1 - e^{k_y \delta z / p^*}) - \dot{z} \approx v_e (1 - e^{(k_y - \delta T) / p^*}) + \frac{\delta T}{k},
\]

where we used the quasi-steady state expression (46) to eliminate the overlap and \( k_y \dot{y} / k \) was discarded since the overlap spring constant typically greatly exceeds that of titin. Averaging and using the isometric constraint yields the tension dynamics

\[
\delta T = -k v_e (1 - e^{(k_y - \delta T) / p^*}).
\]

Note that to determine the tension it is important to include the contribution from \( \dot{z} \), namely the \( \delta T \) term. Indeed the average value, \( \langle \dot{z} \rangle \), is the mean rate of increase in the filament length and omission of this term would miss an important intermediate time behavior in the tension, as will become clear.

The closed system, (47) and (48) is solved to yield \( y \) and \( \delta T \) time profiles. In general this is difficult. Fortunately we can execute this procedure exactly to leading order for the experimentally relevant case of small \( \epsilon \). This is done in the next section.

### 4.5. Slow turnover: small sarcomere variation, \( \epsilon \ll 1 \).

This subsection treats the situation relevant to [2]: slow turnover \( (r < 1) \) and small variation across the fiber, \( \epsilon \ll 1 \). We now solve the equations established in the previous section to leading order in \( \epsilon \).

It will be shown that for all times \( y \sim \epsilon \) is first order, whereas \( \delta T \sim \epsilon^2 \) is second. Now in (45) \( y = y_0 \) is essentially constant, and from (33) \( k_y y_0 \approx \epsilon \bar{f}_s \), neglecting the small turnover term. Thus the tension term can be discarded in the overlap dynamics, (45), giving simple exponential behavior

\[
k \delta z = \epsilon \bar{f}_s (1 - e^{-t / \tau_{\text{olap}}}), \quad \tau_{\text{olap}} = \hat{\lambda} / k.
\]

Using this result for the tension in (45) one gets to leading order

\[
\delta T = -\langle \epsilon^2 \rangle \bar{f}_s e^{-t / \tau_{\text{olap}}} \quad (t \lesssim \tau_{\text{olap}}).
\]

This confirms that the tension deviation is second order during the fast relaxation episode. Tensions change relatively little due to cancellation of the effects of sarcomeres which are, respectively, contractile and expansive relative to the mean.

Note: (i) for \( t > \tau_{\text{olap}} \), since to first order \( k_y y = k \delta z \) from (46) and from (49) \( k \delta z = k_y y_0 \), it follows that \( y \) retains its initial value to this order. This confirms that its relative changes in the early fast phase are small (second order or higher), a requirement for the slow-fast
separation. (ii) Equation (50) is invalid for times beyond $\tau_{olap}$, when the effects of the neglected (de)polymerization terms become important.

Turning to the long time behavior, let us obtain the sarcomere length evolution to leading order from (47). Discarding the second-order tension terms, expanding to order $\epsilon$ and using the initial condition $\kappa_1 y_0 = \epsilon \bar{f}_s$ one has

$$\ddot{y} = -\frac{\kappa_1 y}{\bar{p}^*} e^{-t/\tau_{sarc}} = \epsilon \bar{f}_s e^{-t/\tau_{sarc}}, \quad \tau_{sarc} = \frac{\bar{p}^*}{\kappa_1 \bar{v}_+}.$$  \hspace{1cm} (51)

This is one of our principal conclusions. Since relaxation requires actin filament lengths to attain their equilibrium value, the sarcomere length relaxation time $\tau_{sarc}$ is set by the slow rate-limiting turnover processes. This confirms that the timescale ratio is small, $\tau_{olap}/\tau_{sarc} = k_\ell r/k \ll 1$, justifying the fast–slow separation. Note that in fact our expansion in (47) presumed $\epsilon < \bar{p}^*/\bar{f}_s$ a slightly more stringent condition on $\epsilon$. For example, we estimate $\bar{p}^*/\bar{f}_s \approx 0.25$ for [2].

Finally, let us derive the tension to leading order. Using our result (51) for $y$ in the tension dynamics (48), expanding the exponential and noting the average of all $O(\epsilon)$ terms vanishes one finds the tension obeys a first-order differential equation

$$\dot{\delta}T = -\frac{r\delta T}{\tau_{olap}} + \frac{\langle e^2 \rangle \bar{f}_s^2}{2\bar{p}^* \tau_{olap}} e^{-2t/\tau_{sarc}},$$

after retaining the leading $O(\epsilon^2)$ terms only. This phase begins at $t \gtrsim \tau_{olap}$ when $\delta T$ is close to zero, see (50). Thus we take as initial condition $\delta T(0) = 0$, since $\tau_{olap}$ is a much smaller timescale than those featuring in (52). After assuming $\tau_{olap}/(r\tau_{sarc}) = \kappa_1/k \ll 1$, the solution is the difference of two exponentials

$$\frac{\delta T}{\bar{f}_s} = \frac{\langle e^2 \rangle \bar{f}_s}{2\bar{p}^*} \left\{ e^{-2t/\tau_{sarc}} - e^{-rt/\tau_{olap}} \right\},$$

confirming that the tension deviation is $O(\epsilon^2)$. The time evolution of the tension is interesting: in addition to the expected sarcomere relaxation time $\tau_{sarc}$, a second much shorter timescale $\tau_{olap}/r$ features, intermediate between the short ($\tau_{olap}$) and long ($\tau_{sarc}$) scales. For example, in the experiments of [2] we estimate $\tau_{olap} \approx 1 \text{ s}$, $\tau_{olap}/r \approx 10 \text{ s}$ and $\tau_{sarc} \approx 6 \text{ min}$. The tension is initially a rising exponential involving the shorter timescale, $\delta T \sim 1 - e^{-rt/\tau_{olap}}$. A peak value $\delta T_{\text{max}}$ is then reached after time

$$t_{\text{max}} = \frac{\tau_{olap}}{r} \ln \frac{\bar{r} \tau_{sarc}}{2\tau_{olap}} = \frac{\langle e^2 \rangle \bar{f}_s}{2\bar{p}^*}.$$  \hspace{1cm} (54)

For [2], we predict $t_{\text{max}} \approx 29 \text{ s}$. The late stages, comprising most of the decay, entail exponential fall off $\sim e^{-2t/\tau_{sarc}}$ with time constant one half of the long sarcomere relaxation timescale.

5. Application to stress fiber relaxation experiments

Our model identifies two qualitatively distinct classes of kinetics, depending on the value of the dimensionless turnover rate parameter, $r$. To which class do actual stress fibers belong? We estimate the overlap force where depolymerization is first amplified as $\bar{p}^* = mk_B T/\delta \approx 4 \text{ pN}$, where $\delta = 2.7 \text{ nm}$ is the effective actin monomer size [41]. Here $m = 3$ is the number of actin filaments per minifilament, which we take to be the same as that in muscle [42]. The polymerization velocity is available from turnover experiments in which an actin renewal

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Figure 3. Schematic of sequence of events in the type of experiment studied in the present paper. Blue regions containing myosin alternate with white regions containing α-actinin and $T(t)$ denotes the tension at time $t$. (a) Before $t = 0$, the stress fiber is at steady state with all sarcomeres of approximately equal length and having equal myosin activities. (b) At $t = 0$ a perturbation is applied causing variable numbers of active myosin heads in different sarcomeres. Stronger sarcomeres are represented as darker than weaker ones, with strength relative to the mean characterized by the parameter $\epsilon$. The tension immediately changes to some new value, $T(0)$. (c) Nonuniform myosin strength causes the sarcomeres to start changing length until a new steady state is reached in (d) where the stronger sarcomeres are now shorter than the weaker ones. The tension evolves throughout to enforce a constant stress fiber length.

A timescale of $\sim 5\,\text{min}$ was measured [4]. For the end-to-end treadmilling turnover mechanism employed in our model, this translates into a barbed end polymerization rate that is the sarcomere length, $\sim 1\,\mu\text{m}$ [2], divided by the turnover time, or $u_+ \approx 3\,\text{nms}^{-1}$. Next, we estimate roughly the slope of the myosin force-velocity relation $\bar{\lambda} \approx \beta \bar{f}_s/(2v_{\text{myo}}^0)$, where $v_{\text{myo}}^0$ is the zero-load myosin velocity and the factor $\beta$ accounts for curvature in the relation (see figure 2). Although $\beta$ is unknown for nonmuscle myosin, we use $\beta = 5$, the value for striated muscle [15, 43]. Each myosin molecule can exert $\sim 1.7\,\text{pN}$ of force at stall [44] and each myosin minifilament contains 10–30 myosin molecules [19]. Assuming ten molecules on each side of the minifilament gives $\bar{f}_s \approx 17\,\text{pN}$. We also take $v_{\text{myo}}^0 \approx 300\,\text{nm}\,\text{s}^{-1}$ from gliding assays [45, 46]. Thus we estimate

$$r \approx \frac{\beta \bar{f}_s v_+}{2p^+ v_{\text{myo}}^0} \approx 0.1.$$  \hspace{1cm} (55)

This suggests that real stress fibers will normally follow slow turnover kinetics, the $r \ll 1$ case treated in the previous section.

We now apply our framework to the approximately isometric studies by Peterson et al [2]. This type of experiment is schematized in figure 3. Initially the fiber was in nearly
uniform steady state, all sarcomeres of approximately equal length and having the same level of phosphorylation. At time $t = 0$, the addition of a myosin activator apparently caused an imbalance in the number of myosins phosphorylated in different sarcomeres: sarcomeres near the fiber ends became more activated than those near the center. The peripheral sarcomeres then contracted while those in the center expanded. After some minutes the fiber reached a new steady state.

The data of [2] indicate that $|\epsilon|$ is small because the apparent ratio of myosin activation in end sarcomeres to those in the center was $\sim 1.15$. Our model thus predicts that the fast overlap dynamics will be those of (49), $k \delta z = \epsilon \bar{f}_k (1 - e^{-t/\tau_{\text{olap}}})$ where the overlap relaxation time is $\tau_{\text{olap}} = \tau_{\text{sarc}}/k$. From the discussion above, $\lambda \approx 1.4 \times 10^{-4}$ N s m$^{-1}$. The value of the overlap force constant $k$ is unknown. Assuming overlap builds up a substantial force over some fraction of the full sarcomere length $\sim 1\mu m$, we crudely estimate the critical overlap $z^* = 50$ nm, giving $k = 0.1$ pN nm$^{-1}$. The overlap relaxation timescale is then $\tau_{\text{olap}} \approx 1$ s. Note that provided $k$ is much larger than the titin force constant $k_t$ (required for the relative sarcomere length change to be small during the fast overlap adjustment phase) the sarcomere length dynamics are insensitive to the value of $k$.

Turning to the long time relaxation, since it is likely that the condition $\epsilon < p^*/f_s \approx 0.24$ is satisfied (see figure 5) for the sarcomeres in [2] it follows that the relevant model prediction is given in (51): $y = y_0 e^{-t/\tau_{\text{sarc}}}$ with $\tau_{\text{sarc}} = p^*/(k_t v_s)$. For initially uniform stress fibers, $y_0 = f_s e_{\text{f}}/k_t$ from (33). Taking $k_t \approx 3.75 \times 10^{-3}$ pN nm$^{-1}$ [28], the sarcomere length relaxation timescale is $\tau_{\text{sarc}} \approx 6$ min.

In figure 4, model predictions for sarcomere length versus time are directly compared to experiment. Experimental data is reproduced from figure 4 of [2]. Peterson et al measured the kinetics of central sarcomeres (defined to be within a $10 \mu m$ region about the stress fiber midpoint) and peripheral sarcomeres (defined to be within $7 \mu m$ of a focal adhesion) under the influence of two different myosin activators. These data are compared to our analytical solutions from (51) using $\epsilon$ as a fitting parameter since the full $\epsilon$ profile was unknown in the experiment. All other parameter values were chosen as estimated above. The predicted relaxation kinetics are in good agreement with the experimental data. The differences in the magnitude of expansion may be attributable to differences in how the two myosin activators affect the $\epsilon$ profile or the average amount of myosin in each sarcomere.

To make predictions pertaining to all sections of the fiber, a full $\epsilon$ profile is needed, which can be roughly estimated from the data of [2]. The apparent ratio of myosin activation in end sarcomeres to those in the center was $\sim 1.15$. Assuming a continuous symmetric profile, the simplest possibility is a quadratic profile as shown in figure 5 for a fiber of 50 sarcomeres, a typical stress fiber length. The fluctuations in $\epsilon$ are then $\langle \epsilon^2 \rangle \approx 0.002$ and $\langle \epsilon^2 \rangle^{1/2} \approx 0.04$.

Figure 6 presents numerical solutions for the length dynamics of all 50 sarcomeres in a stress fiber with the $\epsilon$ profile of figure 5 and using all other parameters as defined above. For simplicity we have assumed that all sarcomeres have initial length $1 \mu m$. Analytical solutions are also presented for the most peripheral sarcomere, $n = 1$, and the central sarcomere, $n = 25$. The numerical solutions were obtained by solving the $2N$ coupled ordinary differential equations for sarcomere length and overlap obtained by substituting the expression for the tension, (34), into the $y$ and $z$ dynamics, the first part of (32) and (36), respectively. Note that for the parameter values estimated above, the analytical prediction is very close to the exact numerical solution. Sarcomeres with $\epsilon > 0$ contract, those with $\epsilon < 0$ expand and those with very small $\epsilon$ remain approximately unchanged in length throughout the entire episode, as observed in [2]. This is
Figure 4. Comparison of model predictions for sarcomere length evolution to experimental data reproduced from figure 4 of [2]. Plus signs: experimental data from cells stimulated by calyculin A. Diamonds: experimental data from cells stimulated by LPA. Shrinking sarcomeres are from peripheral regions of stress fibers and expanding sarcomeres from the central region. Solid lines are analytical solutions from (51), $y = y_0 e^{-t/\tau_{sarc}}$, where $\tau_{sarc} = p^*/(k_t v_+) \approx 6 \text{ min}$ and initial sarcomere length was chosen to match the experimental data. $\epsilon$ was used as a fitting parameter. All other parameter values are as estimated in section 5: the minifilament stall force $\bar{f}_s = 17 \text{ pN}$, the myosin internal drag coefficient $\bar{\lambda} = 1.4 \times 10^{-4} \text{ N s m}^{-1}$, the barbed end polymerization velocity $v_+ = 3 \text{ nm s}^{-1}$, the characteristic overlap force $p^* = 4 \text{ pN}$, and the titin spring constant $k_t = 3.75 \times 10^{-3} \text{ pN nm}^{-1}$.

rephrased in figure 7 as a sarcomere length profile evolving in time. Note that under these conditions of small $\epsilon$, our model predicts the sarcomere length profile has at all times the same shape as the $\epsilon$ profile (quadratic here) with only the amplitude changing in time. It would be of great interest to experimentally measure the full sarcomere length profile.

6. Discussion

Model. We developed a model of stress fibers, basic cytoskeletal contractile machines. Based on currently available experimental information, the model assumes a sarcomeric structure. The essential ingredients are myosin contractility, passive elasticity from c-titin, internal resistance forces due to actin filament overlap and actin turnover. The last two are intimately connected by a feedback mechanism in our model: when interference between opposing actin filaments becomes substantial and the overlap force large, depolymerization rates are augmented. Within this broad framework, such a mechanism is inescapable: in its absence, unlimited quantities of actin filament could grow and accumulate in the overlap region in a completely unphysical fashion.
Figure 5. Profile of sarcomere strengths for a stress fiber with 50 sarcomeres. In [2], the degree of phosphorylation in peripheral sarcomeres was \( \sim 1.15 \) greater than that in central sarcomeres, but the full spatial distribution was unknown. Assuming a smooth and symmetric profile, for small enough variation \( \epsilon \) the profile will be quadratic. Using the condition \( \sum_{n=1}^{N} \epsilon_n = 0 \) results in the quadratic \( \epsilon \) profile shown. \( \epsilon \) ranges from \(-0.05\) to \(0.1\) under these conditions. This \( \epsilon \) profile is used in figures 6 and 7.

Figure 6. Model predictions for length evolution of all sarcomeres in a 50-sarcomere stress fiber. Solid lines are exact numerical solutions and dashed lines are analytical solutions for sarcomeres \( n = 1 \) and 25 only. Analytical solutions are from (51), \( y = \epsilon f_s e^{-t/\tau_{\text{sarc}}} \), where \( \tau_{\text{sarc}} \approx 6 \) min. Analytical and numerical solutions are in close agreement. All parameters values are as for figure 4 and the \( \epsilon \) profile is that of figure 5. Note that weak sarcomeres expand (\( \epsilon < 0 \), upper curves) while strong sarcomeres contract (\( \epsilon > 0 \), lower curves). Sarcomeres with myosin activity near the mean value remain approximately unchanged in length.
Figure 7. Model predictions for sarcomere length profile at \( t = 0, 2, 4, 6, 10 \) and 20 min for the 50-sarcomere stress fiber of figure 6. At all times the profile has the same shape as the \( \epsilon \) profile, in this case quadratic; only the amplitude changes. Note that initially all sarcomeres have equal length, \( l = 1 \mu \text{m} \). With time the weak central sarcomeres expand while the strong peripheral sarcomeres shorten. By \( t = 20 \) min, the fiber reaches steady state. For a quadratic \( \epsilon \) profile, the crossovers between shortening and lengthening sarcomeres are at \( n \approx 12 \) and 38.

Model parameters. These physical elements were measured by a set of key model parameters: (i) \( f_s \) and \( \lambda \), the stall force and internal drag coefficient of a myosin minifilament; (ii) the titin spring constant \( k_t \); (iii) the overlap force constant, \( k \); (iv) \( v_+ \), the actin barbed end polymerization rate and (v) \( p^* \), the overlap force above which actin depolymerization is enhanced. Of these, \( f_s, \lambda, k_t \) and \( v_+ \) are directly measurable and we have estimated \( p^* \).

Fast turnover-overlap relaxation, slow sarcomere relaxation. Each of the many sarcomeres comprising the fiber contains oppositely-oriented actin filaments which at some instant have a certain growth rate and degree of overlap. An important feature emerging from the model is that the overlap and filament growth or shrinkage rate relax very rapidly in seconds, while sarcomere lengths relax on a much longer timescale of minutes. During this slow relaxation episode filament growth rates adjust very slowly, determined only by the current value of the slowly changing sarcomere lengths. Thus, the fast polymerization-overlap system is enslaved to the slow sarcomere length system.

Fast turnover kinetics. We find that two qualitatively different classes of stress fiber kinetics are possible, depending on whether turnover is rapid or slow. The class a given system belongs to is determined by the dimensionless turnover rate \( r \equiv \lambda v_+/p^* \). For systems where turnover is fast, \( r \gg 1 \), filament growth rates can adjust rapidly and are compliant to contractile and elastic forces. Sarcomere kinetics evolve as they would if one simply ignored turnover and filament crowding: sarcomere length change results from contractile myosin and elastic titin forces.
working against internal myosin viscosity, with the actin filaments compliantly growing or shrinking as the sarcomere grows or shrinks. Hence the sarcomere relaxation time is \( \tau_{\text{sarc}} = \frac{\lambda}{k_t} \).

The filament growth process offers negligible resistance and does not affect the force balance. During the initial fast phase the actin growth rate is rapidly tuned to match that of the sarcomere by adjusting the overlap, and thereafter the growth rates are maintained equal by continuous slow overlap adjustment in tune with the evolving sarcomere growth rate. The recipe for the overlap to achieve this tuning is the quasi-steady state relation (38) which for small overlap deviations is
\[ k \delta z = (k_t y - \delta T)/r. \]

Using (41) and noting filament growth rate equals \(-v_+ \delta z/z^*\), the quasi-steady state relation translates to an explicit equality of filament and sarcomere growth rates,
\[ v_+ + v_- = -\dot{y}. \]

Real stress fibers: slow turnover kinetics. We estimate that real stress fiber systems which have been studied experimentally belong to the slow turnover class, \( r \ll 1 \). The behavior is very different since polymerization kinetics are now sluggish and adjustment of actin growth rate requires substantial forces. Myosin and titin forces now determine the filament growth rate not via the sarcomere growth rate, but by a direct force balance: the overlap force matches the myosin and titin forces. There is sufficient time to establish this force balance before filament growth or shrinkage since turnover is slow. Once the overlap force is determined, this determines the filament growth rate which depends only on overlap. The sarcomere can then only change length as fast as the filaments themselves can change length; hence the sarcomere growth rate is equal to this slow rate-limiting filament growth rate. The early fast relaxation episode consists in the filament rapidly translating at fixed length until the overlap matches the myosin and titin forces. Thereafter the overlap maintains this force balance by slow adjustments; this is the quasi-steady state relation
\[ k \delta z = k_t y - \delta T, \]

which determines the filament and sarcomere growth rate \( \dot{y} = -v_+ \delta z/z^* \approx -v_+(k_t y - \delta T)/p^* \) taking small overlap as an example. This result shows how the sarcomere relaxation time results from titin elastic forces acting on the polymerization-overlap system to determine filament growth or shrinkage. The polymerization-overlap system has an effective internal ‘drag coefficient’ equal to \( p^*/v_+ \), since an imposed force \( p^* \) generates a response in filament growth rate of order \( v_+ \). Thus the relaxation time is
\[ \tau_{\text{sarc}} = \frac{p^*}{k_t v_+}. \]

This is one of the model’s principal predictions.

Experimental stress fiber systems. We applied our model to the experiments of Peterson et al [2] in which sarcomere expansion and contraction was observed after myosin stimulation. For such stress fiber systems we estimate the dimensionless turnover parameter \( r \approx 0.1 \), squarely in the slow turnover class. For [2] we estimate \( p^* = 4 \) pN and used the muscle titin value \( k_t \approx 3.75 \times 10^{-3} \) pN nm\(^{-1}\) [28], while \( v_+ \approx 3 \) nm s\(^{-1}\) is available from experimental actin turnover measurements [4]. Using these in (56) gives \( \tau_{\text{sarc}} \approx 6 \) min, close to the observed relaxation time. Note that even though several model parameter values (\( f_s \), \( \beta \) and \( k_t \)) were estimated from striated muscle, \( \tau_{\text{sarc}} \) differs from muscle sarcomere relaxation times which are typically on the order of seconds [47, 48]. This is as expected since the stress fiber mechanisms articulated by the present model are quite different to those believed to govern muscle. Whereas we predict that actin filament length change is the rate-limiting process in stress fibers, it has been proposed that in striated muscle actin filament length is maintained constant by the molecular ruler nebulin [49].
or by the activity of the pointed end capping protein tropomodulin [50]. Moreover, in striated muscle actin does not undergo treadmilling [50] in contrast to stress fibers [31].

**Effect of sarcomere variation amplitude.** An important aspect of the relaxation kinetics is the extent of myosin activity variation along the fiber from differential stimulation. The relative variation is named $\epsilon$ in our model. For the experiments of [2] this was apparently small, with $|\epsilon|$ likely to be considerably less than unity for all sarcomeres. For this case we find that sarcomere length changes during relaxation are $\mathcal{O}(\epsilon)$ whereas tension changes are much smaller, $\sim \mathcal{O}(\epsilon^2)$. Our model as phrased here is geared for relatively small $\epsilon$; we note that for larger variations, $\epsilon > 0.2$, it generates unphysical negative sarcomere lengths. In reality, when a sarcomere becomes as short as the length of a myosin minifilament, $\sim 0.3 \mu m$, minifilaments from adjacent sarcomeres would abut and prevent further contraction.

**Role of cellular titin.** We find that a force that responds to sarcomere length is necessary in order for a stable steady state sarcomere length profile to be possible. The discovery of c-titin in stress fibers [14] strongly suggests titin fulfills this role, and further emphasizes the similarity between the well-known sarcomeric structure of muscle and that of stress fibers. It is possible that titin has additional and more complex roles. A kinase domain that may actively respond to force in muscle is present in c-titin [14].

**Actin turnover mechanism and its regulation.** Turnover regulation is necessary in order that a steady state be accessible but the precise mode of turnover and the mechanisms regulating it are unknown. Our model invokes a simple feedback mechanism whereby pointed end overlap promotes pointed end dissociation. Other possible mechanisms include regulation of the severing protein cofilin which may play an important role in actin disassembly as suggested by recent experiments using cofilin knockdown cells [34]. It has been suggested that cofilin’s actin binding mechanism [51] could make its activity force-dependent [52]. Another possibility is that the pointed end depolymerization rate is fixed and regulation occurs instead through force-sensitivity of nucleators at the polymerizing barbed end, possibly formins [52]–[54]. The detailed behavior depends on structural details near the barbed end and is beyond the scope of the present paper. For example, if one assumes the titin connects to a Z-line-like region but not directly to actin filaments, as in figure 1, we find a timescale $\tau_{\text{sarc}} = p^*/(k_t v_0^0)$ where $p^*$ now denotes the threshold value of the force tending to pull the nucleator away from the barbed end above which the actin association rate is strongly augmented. Whatever particular mechanisms may turn out to be operative, our model has attempted to articulate certain quite general features. For example, the relaxation time of (56) may have broad significance provided the characteristic turnover rate $v_+$ and threshold force $p^*$ are appropriately reinterpreted. Future and more detailed experimental studies of stress fiber kinetics promise to shed light on these important questions.

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