

Nonlinear contractile response of actomyosin active gels to control signals

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Biological systems tightly regulate their physiological state using control signals. This includes the actomyosin cytoskeleton, a contractile active gel that consumes chemical free energy to drive many examples of cellular mechanical behavior. Upstream regulatory pathways activate or inhibit actomyosin activity. However, the contractile response of the actomyosin cytoskeleton to control signals remains poorly characterized. Here, we employ reconstituted actomyosin active gels and subject them to step and pulsatile activation inputs. We find evidence for a nonlinear impulse response, which we quantify via a transfer function $\delta\epsilon/\delta g$ that relates input free-energy pulses δg to output strain pulses $\delta\epsilon$. We find a scaling relation $\delta\epsilon/\delta g \sim g^{-0.3}$. The negative sign of the exponent represents a decreased effectiveness of a contracting gel in converting energy to strain. We ascribe nonlinearity in our system to a density-dependent mechanism, which contrasts strain-stiffening nonlinear responses to external stresses. Contractile response to control signals is an essential step toward understanding how information from mechanical signaling processes flows through actomyosin networks in living, and likely also synthetic, cells.

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I. INTRODUCTION

Cells are micron-scale acrobats, capable of remarkable mechanical tasks such as crawling, dividing, and healing wounds. To describe these tasks, simple mechanical models often suffice [1]. But mechanics alone are not enough. Cells also rely on intracellular biochemical signaling proteins, which constitute an information-processing “circuit” that drives gene expression and thus decision-making within and across cell cycles [2]. A well-studied example of signaling in a mechanical context is mesenchymal stem cells, whose fate is sensitive to mechanical information [3,4]. A tight orchestration between mechanics and signaling is required to coordinate proper cellular acrobatics and, more broadly, survival.

One key driver of cellular mechanical behavior is the actomyosin cytoskeleton. Its mechanical properties and ability to contract have been well studied [5]. Equally important is its interactions with biochemical signaling pathways. Actomyosin activity responds to upstream signals that are mediated by Rac1 and RhoA [6] and calcium [7]. Meanwhile, actomyosin forces contribute to downstream signals triggered by mechanosensitive proteins such as filamin [8], integrin complexes [9], and ion channels [10]. Furthermore, actomyosin tension affects the binding affinity of accessory

proteins, potentially also contributing to intracellular signaling [11]. Finally, mechanical information influences several key cellular pathways, including Wnt [12], YAP/TAZ [13], and Hippo [14]. In all, actomyosin contractility is deeply embedded in the intracellular signaling circuitry. But how does contractility participate in information flows within this circuit?

Control theory and information theory have offered powerful quantitative frameworks for describing intracellular information flows. A well-researched example is sensing chemical environments. Examples include chemotaxis [15], quiescence/growth [16], and quorum sensing [17]. In these examples, the response (gene expression) to signals (environmental ligand binding) is well understood. Meanwhile for the actomyosin cytoskeleton, the contractile response of actomyosin contractility to activation signals remains less understood. Characterizing this response is necessary to understand how information flows through the actomyosin cytoskeleton in the same way it flows through signaling proteins subject to reaction-diffusion dynamics.

Here, we perform experiments with reconstituted gels to determine the contractile response of actomyosin active gels to control signals. We first investigate gels under an effectively constant activation signal (step response) and observe fast dynamics to a densely contracted configuration. Remarkably, the gel consumes chemical free energy well after contraction ceases. We develop a linear hydrodynamic model and find disagreement with our observations. This comparison suggests that the gel’s response to motor activity depends on density, leading to nonlinear contractile properties. In order to determine contractile properties, we next investigate pulsatile activation signals (impulse response). Pulsatile contractility has been widely reported across cells [18], yet many experimental studies on actomyosin contractility investigate step

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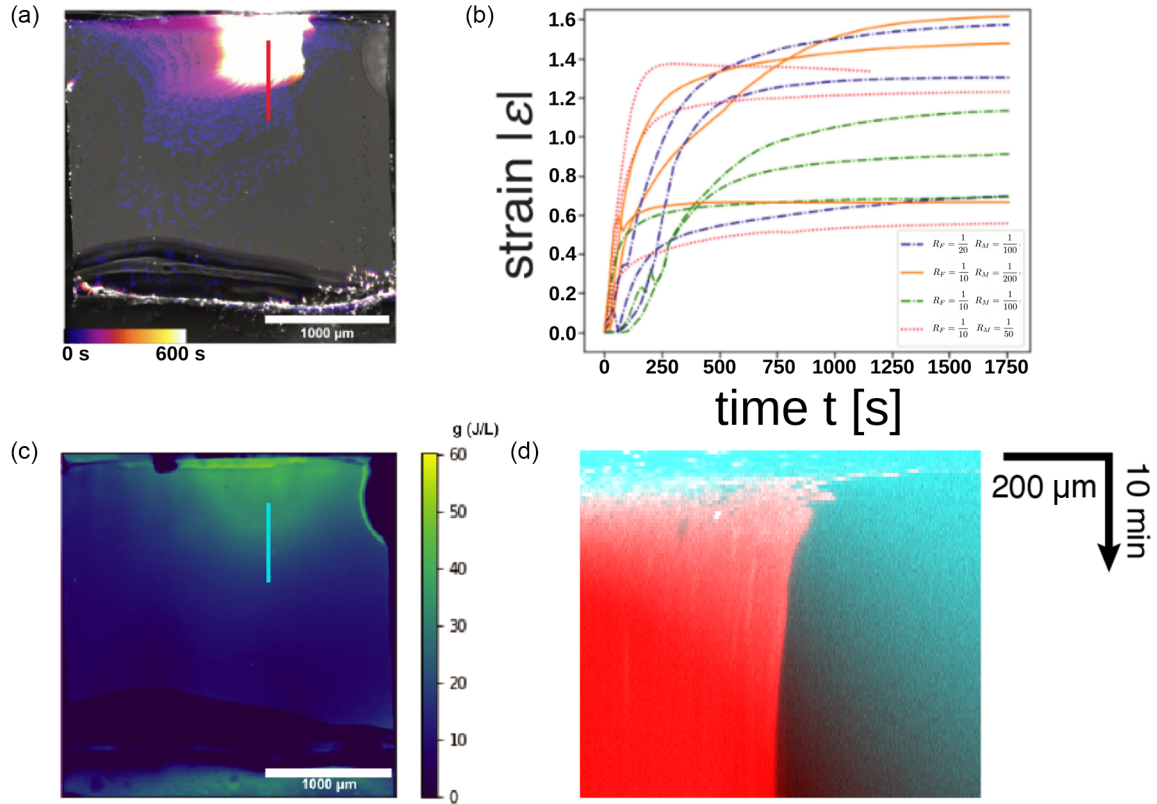


FIG. 1. Step input activation of actomyosin contraction. (a) Color-time overlay of a contraction event ($[\text{actin}] = 12 \mu\text{M}$, $R_M = 1/50$, $R_F = 1/10$). Color corresponds to time after sample preparation (calibration bar, lower left). (b) Strain trajectories $\varepsilon(t)$ for 12 independently prepared samples. (c) Spatial distribution of cumulative Gibbs free energy consumed g , determined by nicotinamide adenine dinucleotide (NADH) assay. (d) Kymograph taken over a column of pixels from the actin (red) and NADH (cyan) images [red line, panel (a); cyan line, panel (c)]. The NADH kymograph exhibits a wave of NADH defluorescence emanating from the contracted gel.

responses. Furthermore, an advantage of impulse responses is that they directly probe the system dynamics [19]. We report a nonlinear contractile energetic compliance $\delta\varepsilon(g)/\delta g$, with a negative scaling with the free-energy density g consumed by the gel. The negative sign of this exponent indicates that as the gel deforms in response to an input of energy δg , the deformation to the next energy input δg will decrease. Nonlinear contractility may lead to concrete advantages. We discuss how pulsatile activity maximizes the energetic cost of contraction. We also discuss the actomyosin as a conduit of signals and mutual information.

II. RESULTS

A. Step response

In order to quantify the contractile response of actomyosin active gels to control signals, we turn to reconstituted active gels of actin filaments, myosin motors, and fascin cross-links. We first consider a common scenario: contraction in the presence of a constant supply of ATP. This scenario mimics the intracellular environment and is straightforward to implement in reconstituted systems by including enzymes that regenerate ATP [20]. We prepare contractile active gels, load onto observation chambers, and record contraction events using fluorescence microscopy [see methods in the Supplemental Material [21]; Fig. 1(a)]. We determine each sample's strain trajectory $\varepsilon(t)$ by tracking the gels' imaged area and com-

puting strain [see methods in the Supplemental Material [21]; Fig. 1(b)]. We observe that strain rises from an initial value of zero to some finite, steady value, with $\varepsilon_f = 1.10 \pm 0.32$ across all trials. Strain rises within a characteristic timescale $\tau = 220 \pm 130$ s. The strain trajectory appears to resemble an exponential rise $\varepsilon(t) = 1 - e(-t/\tau)$, which is the solution to a first-order linear differential equation. This resemblance appears to suggest that contractility agrees with a linear model.

In order to test this suggestion, we apply a linear model

$$0 = K\varepsilon + \eta\dot{\varepsilon} + J,$$

where K is the bulk modulus, η is the effective viscosity, and J is a constant active stress exerted by the ensemble of myosin motors. The solution $\varepsilon(t)$ of this equation is given by an exponential rise, with $\varepsilon_f = J/K$ and timescale $\tau = \eta/K$. We plug in values based on prior studies to determine agreement between the linear model and experiments. With $K \approx 1$ Pa [57] (assuming that bulk and shear moduli are comparable), $\eta \approx 1$ Pa s [58], and $J \approx 30$ Pa [59], we have $\varepsilon_f \approx 30$ and $\tau \approx 1$ s. Comparing these values to our experimental results reveals a discrepancy of two orders of magnitude: Our gels deform less and deform more slowly than predicted by the linear model. What is the origin of this discrepancy?

Characterizing the response of active gels to motor activity is experimentally challenging. Measuring the response to external shear is straightforward, because both shear stress σ and shear strain γ can be simultaneously measured with

a rheometer to yield the shear modulus $G = \sigma/\gamma$. Meanwhile, in active gels, measuring the active stress J is more challenging than measuring the bulk strain ε . A few studies have experimentally estimated active stresses for actomyosin [59] and microtubule-kinesin active gels [60]. Rather than estimating active stress here, we leverage an established assay to measure consumption of chemical free energy by the myosin motors [61–63]. In short, we couple ATP consumption to the oxidation of NADH using two enzyme-substrate pairs. Since ATP hydrolysis is stoichiometrically coupled to NADH oxidation, loss of NADH fluorescence reports free-energy consumption. This method allows us to measure the spatial distribution of free-energy consumption while measuring strain simultaneously [Fig. 1(c)].

In active systems, the chemical potential difference $\Delta\mu$ —often arising from ATP hydrolysis in biological systems—drives active processes. Linear hydrodynamic theory gives us the active stress relation, which is defined as $\sigma_a = \zeta \Delta\mu$. Similar to viscosity, the activity coefficient ζ is a material property of the cytoskeleton, which characterizes both the activity of the motors and the ATP consumption. For the limit, when we assume ζ as a constant, active stress contribution is constant as J . Since the total amount of free energy consumption per unit volume is given by $g = \Delta\mu \times c$, where c is the concentration of active elements (e.g., motors, ATP-consuming sites), we can establish that $\sigma_a \approx \zeta g$. This relationship reflects how the active stress arises directly from the energetic consumption processes in the system. By combining these principles, the relationship $J \sim g$ reflects how active stress scales with the rate of free-energy consumption, aligning with established models in active gel theory [64,65].

This leads to the expectation that the active stress J is proportional to the total free-energy density consumed: $J \sim g$. If this assumption were correct, we would expect a constant, finite amount of free energy consumed by the gel, and this consumption could likely occur over the timescale $\tau = 250$ s of contraction. To test this expectation, we plot a kymograph of the NADH concentration near the site of the contracted cluster [Fig. 1(d)]. We observe that NADH continues to be oxidized well past the characteristic contraction timescale τ , in contrast with our simple expectation. We instead observe a wave of oxidation emanating from the contracted gel. This wave is likely governed by reaction-diffusion dynamics. To confirm, we simulate our experiments by numerically solving for the governing reaction-diffusion dynamics, incorporating a Michaelis-Menten model of reaction kinetics, diffusion of reagents, deformation of the gel, and the assumption that the ATPase rate k_{myo} of the gel is constant. We fit the outputs of multiple reaction-diffusion simulations, allowing k_{myo} and the effective diffusion constants to vary, compare the resulting NADH concentration fields to our data, and minimize the error using the Nelder-Mead method to extract the myosin ATPase rate. Overall, we find good agreement between data and simulation [Figs. 2(a) and 2(b)], with $k_{\text{myo}} = 10^{-4}$ mM/s. This fit confirms that $\dot{g} \approx \text{const}$, with a typical scale of $\dot{g} \approx 5$ W/m³. These results are largely consistent with recent measurements [63].

To summarize, we have experimentally found that (1) strain rises toward a terminal value within a finite time, (2) free energy is continuously consumed well past this time,

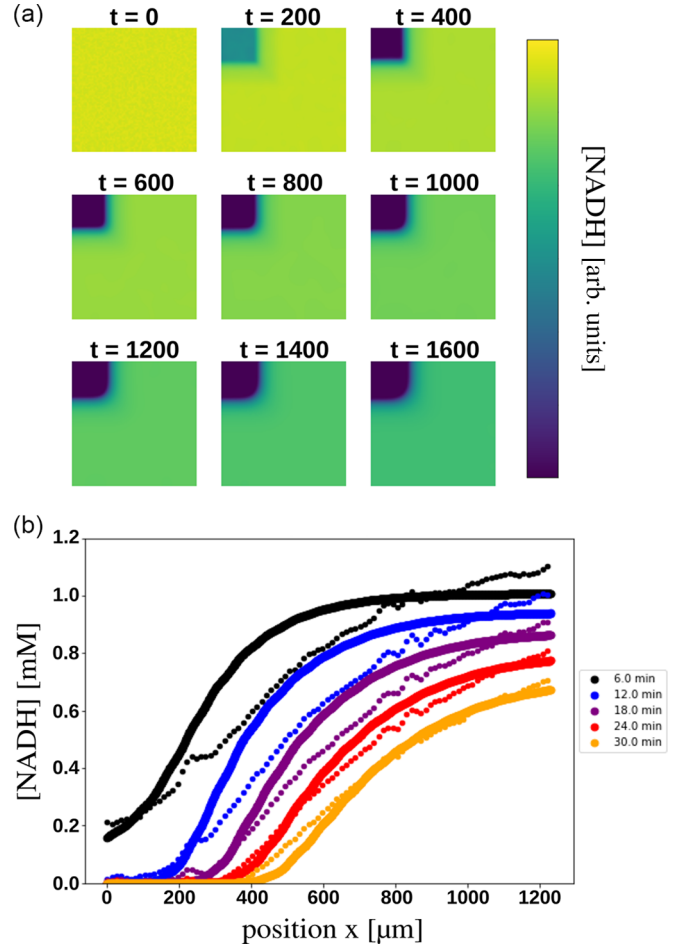


FIG. 2. Reaction-diffusion dynamics confirm a constant chemical free-energy consumption rate. (a) Snapshots of the NADH concentration field for nine sample times: $t = 0$ s (top left) through $t = 1600$ s (bottom right), in steps of 200 s. Color corresponds to NADH concentration, normalized to its initial value (calibration bar, right, in arbitrary units). (b) Plot of NADH concentration vs space (abscissa) and time (color; legend, right) for experiment (points) and simulation (bold line).

and (3) a linear model fails to predict values for ε_f and τ . Furthermore, the observation $\dot{g} = \text{const}$ appears to be at odds with $J \sim g$. If both relations were true, active stress would continue to increase with time. Here, we consider one modification to our linear model that may reconcile both observations: a density-dependent nonlinearity. As the gel contracts, the density of actin as well as myosin motors increases. We presume this increased density affects K , η , and J , thus making them functions of the output strain ε . (We note that in the actomyosin cortex of living cells, actin filaments undergo rapid turnover; nonlinearity in K could thus be excluded *in vivo*; see Sec. III). Indeed, density-dependent mechanisms have been discussed in active gel models [64,66–68] but remain poorly characterized experimentally. It may be likely that our step-response data could probe density-dependent nonlinearity. Indeed, an exponential fit to our strain trajectories reveals systematic residues, indicating the presence of additional effects not captured by a linear model. However, measuring density-dependent nonlinearity with a

step response is difficult, because deviations from linearity manifest primarily in transients. Probing nonlinear responses in externally driven materials is possible with equipment such as rheometers, which can drive the system sinusoidally with a single frequency and systematically vary that frequency. Active gels cannot be driven “backward” because active stresses primarily cause contraction, and not extension. Thus, in order to systematically probe the nonlinear contractile response of actomyosin, we turn to a different kind of driving.

B. Impulse response

In biology, actomyosin contractility is rarely activated for sustained periods of time. Researchers have long documented the observation that contractility proceeds in small, spatially and temporally localized “bursts” and “waves,” collectively termed “pulsatility” [18,69,70]. Despite this biological relevance, it remains poorly understood how active gels contract in response to pulses in activity.

We drive actomyosin gels impulsively using an assay including caged ATP (see methods in the Supplemental Material [21]). This analog of ATP remains protected from hydrolysis until illuminated with UV (365 nm) light, which induces an “uncaging” reaction, thereby making the ATP available for hydrolysis. We prepare the biochemical composition of these gels identically (see methods in the Supplemental Material [21]); we instead vary across experiments the pulse width, τ_e , between 50 and 200 ms. We chose this range because we found that 50 ms pulses are short enough to liberate a small fraction of caged ATP, while 200 ms pulses uncaged a majority of all caged ATP molecules. We wait 900 s between pulses, which is significantly longer than the rise time of $\tau^* = 250$ s we observed in the step assay. We again use fluorescence microscopy to observe the contraction resulting from each pulse. Figures 3(a) and 3(b) demonstrate the discrete contractile events that result from individual activation pulses—where panel (a) provides a time-color overlay and panel (b) plots the strain evolution as a function of time, $\varepsilon(t)$, as shown by the orange curve. The individual pulses (vertical blue lines) differ from the step response: Rather than immediately rising to a single terminal strain ε_f , each pulse releases a small packet δg of energy that ultimately results in a small increase $\delta\varepsilon$ of strain [Fig. 3(c)].

To determine δg , we develop an analytical model accounting for Michaelis-Menten kinetics (see methods in the Supplemental Material [21]). The results of this model are represented in Fig. 3(d), where it is in good agreement with the $\delta\varepsilon$ data shown in Fig. 3(c). We keep the pulse duration constant within an experiment. However, each pulse releases ever fewer caged ATP molecules and thus δg per pulse decreases. This is because this assay lacks ATP regeneration as in the step assay. After several pulses, gels in the pulse assay approach an ultimate terminal strain ε_f [Fig. 3(b), horizontal green line]. We note that the values of terminal strain observed in our pulse assay are lower than those in the step response. We carefully choose our starting concentration of caged ATP to be sufficiently low to exclude steric repulsion from close packing of actin, which likely plays a significant role once the gel is fully contracted.

Although there appears to be a linear relationship between strain and energy consumption, we ask whether the dynamics are indeed governed by a linear contractile compliance. In order to answer this question, we take inspiration from a quantity developed in nonlinear rheology: the differential modulus $\delta\sigma/\delta\gamma$ [71]. In analogy, we ask whether the differential contractile compliance, $\delta\varepsilon/\delta g$, is linear or nonlinear.

To answer this question, we first turn to the frequency dependence of ε in Laplace space [Fig. 3(e)]. We observe a functional form consistent with that of a low-pass filter, expected of many dynamical systems [19]. We fit to the functional form $\varepsilon = \frac{\varepsilon_f}{1+s^\xi}$, where s is a Laplace frequency scaled by the filter’s corner frequency, ξ is a scaling exponent, and ε_f is the terminal strain or zero-frequency response known as the dc gain. We obtain values for ξ of $\xi_{50} = 1.801 \pm 0.055$ and $\xi_{200} = 1.682 \pm 0.022$. These values of ξ indicate that the strain behavior of the system is not a linear first-order system, as expected for the case when $\xi \neq 1$. However, we note that these observations for ξ do not necessarily suggest that the system is nonlinear.

To further test for linearity, we investigate the dependence of the compliance, $\delta\varepsilon/\delta g$, on g . This quantity is built through determining the value of $\delta\varepsilon/\delta g$ using Laplace transformed data for each pulse. If this relationship were linear, it would have no dependence on g . However, we indeed find a scaling relationship $\delta\varepsilon/\delta g \sim g^\zeta$, with $\zeta = -0.316 \pm 0.128$ [Fig. 3(f)]. The negative sign of the scaling exponent ζ reflects diminishing returns: As the gel contracts in response to a packet δg of energy, it contracts less when receiving an additional packet δg .

III. DISCUSSION

A. Potential mechanisms underlying nonlinearity

We have quantified the contractile response of contractile active gels to two kinds of input signals: step signals and pulsatile signals. For the case of the step signal, we identified a saturation nonlinearity that sets in above a certain threshold energetic input. This is similar to other nonlinearities, such as with shear stiffening of biopolymer networks [71–73] or shear thickening/thinning of non-Newtonian fluids. In our experiments, the underlying mechanism that changes past this critical energetic input is motor slip, as evidenced by the continued consumption of ATP after contraction has ceased.

For the case of pulsatile signals, we chose the overall caged ATP concentration low enough to exclude this saturation nonlinearity. One can see this in Fig. 3(b), where the maximum strain we attain in the pulse response experiments (~ 0.4) is lower than that achieved in the step response case (~ 1 , cf. Fig. 1(b)). We found that the impulse response, characterized by the quasistatic transfer function $\delta\varepsilon/\delta g$, varied with the total amount of free energy density g consumed by the gel [cf. Fig. 3(f)]. One would have expected a linear response here, given the small inputs. This nonlinear response demonstrates that the contractile properties of actomyosin contractile gels change as they contract. The mechanism we put forward is that a single pulse increases not just cross-link density (and hence bulk modulus) but also motor density (and hence the active stress that causes strain). Our mechanism argues that

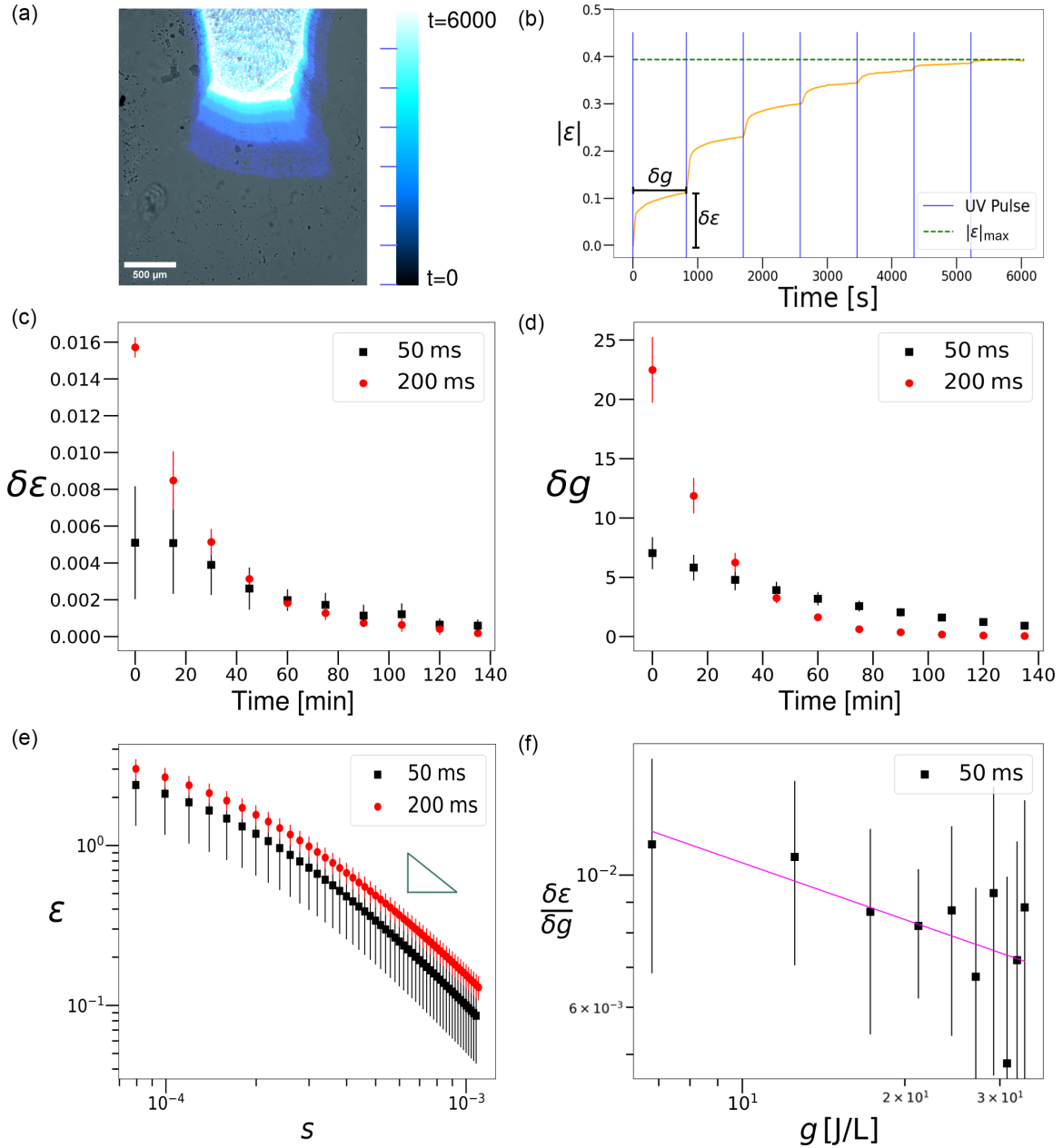


FIG. 3. (a) Color-time overlay of epifluorescence micrographs. Color corresponds to time as shown in the color bar to the right. Scale bar = 500 μm. (b) Absolute value of strain vs time for a representative sample. Blue vertical bars in overlay represent UV pulse inputs that correspond to the blue horizontal lines alongside the color bar in panel (a). Maximum strain achieved in this sample is indicated by the horizontal dashed green line. (c) The change in strain, $\delta\epsilon$, as a function of time t shown for $\tau_e = 50$ ms (black squares) and $\tau_e = 200$ ms (red circles). (d) The change in the amount of consumed free energy δg as a function of time t shown in the same format as panel (c). (e) Laplace transformed strain ϵ vs Laplace frequency s , shown in the same format as panel (c). Inset right triangle indicates the high-frequency scaling with $\xi_{50} = 1.801 \pm 0.055$ and $\xi_{200} = 1.682 \pm 0.022$. (f) $\delta\epsilon/\delta g$ vs cumulative free energy density g shown on log-log axes. Error comes from $N = 3$ identically prepared replicates. Magenta line indicates the linear fit of the data with a slope of $\zeta = -0.316 \pm 0.128$ ($P = 0.014$ compared to zero).

an imbalance in these two density dependencies results in a transfer function that changes as contraction proceeds, hence nonlinearity. In quantifying responses to energy consumption (and, by inference, to active stress) from internal myosin ATPase activity, we argue that this response is more closely aligned with the actomyosin cytoskeleton's primary function of generating tension and shape change. Density-dependent

nonlinear properties were considered in prior theoretical studies [66,67] and were the underlying factor in contractile instability.

We propose a simple picture to explain the density-dependent nonlinear response of contractile actomyosin gels. We regard the actin filaments of the gel comprising two populations: the *tension-bearing backbone* and the *residual*

background network. During the initial stages of contraction, many actin filaments undergo tension from myosin motor activity. Prior studies have shown that motors bend, buckle, fragment, and compact actin [74,75]. Therefore, we anticipate that as contraction proceeds, actin filaments gradually transition from a tension-bearing state (before being processed by myosin) to a space-filling medium that no longer bears tension. When contraction ceases, the terminal strain is likely determined by a balance between active stress J of the remaining tension-bearing backbone and the bulk compressibility K of the residual background network. A recent study has demonstrated that interfilament contacts contribute significantly to external compression in actin systems, similar to sheep's wool [76]; we thus expect that K is set by such interfilament contacts. Furthermore, J is likely subject to additional effects beyond the motor distance, including transverse interfilament tension propagation [77]. It would be interesting to study how these two concurrent mechanisms give rise to nonlinear response. However, directly measuring active stress remains an experimental challenge, and accurately extracting thermodynamic quantities like mechanical work and power remain a challenge.

Additional physical mechanisms may also contribute to density-dependent nonlinear contractile response. Cross-links of different types affect the architecture of the actin network [78], which in turn affects the response to external stress [79] and myosin energy conversion to work [63]. Additionally, our model does not account for dissipation due to elastic stress relaxation [80], plasticity [81], or prestress within the network, which has been shown to affect the slope of the storage modulus, G' , at low frequencies (see the Supplemental Material [21]) [82]. Additionally, our study assumes that the coupling between free energy g and active stress J is constant. Although this is a common assumption, its validity is difficult to test experimentally. Molecular motors deliver limited power, which is captured in the force-velocity curve. Furthermore, assemblies of motors, such as muscle and myoblasts, can exhibit a nonlinear force-velocity curve and force-dependent ATPase activity [83,84], which can result from a nonlinear coupling between myosin unbinding and force/velocity [85,86]. Our calculations show that each myosin motor bears approximately 0.1 pN of force during pulse response experiments. (We arrive at this result by noting $K = J/\varepsilon$ and $Nf = F = JA$, with $K = 1$ Pa the bulk modulus, J the active stress, $\varepsilon = 0.4$ the magnitude of the maximum strain from the pulse experiments, $N = Vc$ the number of myosin motors in the gel, $V = 0.9$ mm³ the volume of sample, $c = 0.12$ μ M the myosin concentration, f the force per myosin, F the total force applied on the gel's boundary, and $A = 20$ mm² the surface area of the gel. We obtain $f = \frac{K\varepsilon A}{cV} = 0.1$ pN.) This force is small compared to myosin's stall force of 6 pN. We conclude our pulse contraction experiments are operating close to the no-load limit of the force-velocity curve, where force-dependent effects on myosin's sliding velocity or ATPase rate can be neglected. We do anticipate, however, more pronounced force-dependent effects if the actomyosin gel were coupled to a rigid or deformable surface, or if the gel is maximally contracted as in the step response experiments. These physical mechanisms highlight the need for methods that measure stress and strain simultaneously and independently of each other.

Finally, we note that the term “nonlinear” is highly utilized and may refer to many different effects. For instance, there is nonlinear mechanics, describing the transfer function from stress to strain. There is also nonlinear mechanochemistry, describing the transfer function from energy consumption to stress transfer function. There is also nonlinear signal transduction, which refers to the transfer function from activation to energy consumption. Although our mechanical model puts forth an established mechanism that is consistent with our experiments, we cannot rule out the combination of these other effects. Additionally, one would expect linear response at arbitrarily small inputs, which would be interesting to test experimentally.

B. Extending to cells, tissues, and robots

One difference between our *in vitro* assay and the actomyosin cytoskeleton of living systems lies in dynamics and adaptability. The actomyosin cytoskeleton is highly dynamic and undergoes constant assembly, restructuring, and disassembly dependent on the mechanical task. Actin filaments *in vivo* undergo turnover over timescales of a few minutes [87], allowing for faster stress relaxation than our *in vitro* assay. We expect that any residual background network *in vivo* would undergo rapid turnover. This would result in a negligible bulk modulus K . We would therefore expect a scaling relation $\delta\varepsilon/\delta g \sim g^\zeta$ with some positive scaling exponent ζ *in vivo*. The positive sign would reflect the fact that active stress J increases as contraction increases motor density.

However, for timescales shorter than actomyosin turnover, we may still expect a negative sign for ζ . We have shown that the transfer function $\delta\varepsilon/\delta g$ is maximal in the limit $g \rightarrow 0$ due to the negative sign of the exponent ζ in the scaling relation $\delta\varepsilon/\delta g \sim g^\zeta$ [cf. Fig. 3(f)]. This is because an influx of energy δg causes an increase in density, which affects the gel in a way that makes future supplies of energy δg less effective: The negative sign of ζ therefore represents diminishing returns. The transfer function $\delta\varepsilon/\delta g$ has an alternative interpretation: It quantifies the energetic cost of contraction, akin to economy measures like cost of transport [88]. In this context, our results suggest that energy economy is maximized in cells when the cytoskeleton is activated with pulsed signals, as opposed to step signals (provided that pulses occur less frequently than the timescale of turnover of the actomyosin cytoskeleton).

Understanding the actomyosin cytoskeleton's response to control signals is an essential step in understanding information flows in cells. The actomyosin cytoskeleton embeds controller and sensor elements that facilitate a flow of mechanical signals [4]. This includes RhoA, which coordinates actin polymerization through formin activation [89,90]. Another, ROCK, phosphorylates nonmuscle myosin to regulate its activity [91]. Further, mechanosensitive proteins trigger downstream signal cascades when stretched [92,93]. Such components likely endow cells with the controllability and observability needed to establish what is closer to full-state feedback control [19].

In our model system, which lacks the precise biochemical regulators present in cells, we are instead regulating activity via the fuel source. It is important to note that we do not include the important force-dependent ATPase activity in this

study [22]. This omission is due to the fact that we cannot measure force or stress in our experiments, and thus cannot reliably test hypotheses that pertain to force-dependent ATPase activity. However, we do consider the decrease in myosin ATPase activity at lower ATP concentrations (see methods in the Supplemental Material [21]). Furthermore, our work has accomplished something not found in prior studies: subjecting a contractile actomyosin gel to multiple pulses of activation. Previous experiments on contractile actomyosin that explored control relied on blebbistatin [23,24], which provides a one-time switch. More recent experiments with noncontractile actomyosin liquid crystals based on myosin IX constructs have established reversible optogenetic control of the motors directly [25]. However, to our knowledge, this technique has not been extended to contractile gels. We therefore have resorted to controlling activity via ATP for lack of a better alternative to establish repeatable temporal control of system activity. However, the use of ATP does result in the formation of strongly bound rigor bonds that form upon ATP depletion between consecutive pulses. This is due to our use of skeletal muscle myosin, which bind strongly to actin in the absence of ATP. In living cells, unphosphorylated nonmuscle myosin is not bound to actin. This effect may contribute to nonlinearity in addition to the mechanism we have put forward, and we highlight this as a key difference between our model assay and a living cell. We also note that researchers studying skeletal muscle myosin *in vitro* have long established 0.1 mM ATP as an optimal concentration that promotes a high duty ratio; increasing ATP to mM levels does not result in faster velocity, but it does lead to more time spent in the weakly bound ATP state and therefore processivity decreases [26,27].

In cells, different mechanisms underlie contractile instability. For *in vivo* actomyosin systems, spatial gradients in tension result in advective flows and contractions that lead to tissue shape changes [28,29]. This is another point of difference between our *in vitro* model system and many *in vivo* systems. Whereas an active fluid description is suitable for *in vivo* actomyosin systems due to the fast timescales of actin filament depolymerization, our *in vitro* gels are more elastic. Previous studies have attributed contraction in these systems to the asymmetric response to tension and compression [30,74]. Furthermore, *in vitro* actomyosin gels have a rich microstructure with myosin foci connected by actin networks and bundles [31].

It is also important to consider the role of feedback effects that influence tension generation. Indeed, recent work on *Drosophila* embryos demonstrated feedback between strain and tension activation [32]. This results in nonlinear mechanochemistry and self-organized patterns that we do not address in our study. However, our study does begin to quantitatively investigate the relationship between pulsatile tension activation and the resulting strain, which has been experimentally poorly characterized. We report a nonlinear response in our model system. However, based on key differences highlighted above, it remains unknown whether a similar nonlinearity holds in the actomyosin cytoskeleton of living cells.

The actomyosin cytoskeleton can be viewed not only as a generator of force, but also as a conduit of mechanical information from upstream regulatory pathways to downstream mechanosensitive cascades. This view is emerging in similar active and biological systems [33–35]. Control over contractility and signal throughput in reconstituted actomyosin active gels will therefore not only shed light on cellular processes, but could also be used in designs for micron-scale robots, living materials, or synthetic cells [36–38].

IV. CONCLUSION

In this study, we have leveraged concepts from control theory to study and characterize the role of variable inputs in contractile actomyosin systems. We demonstrate the presence of inherent nonlinearities for step and quasistatic impulse inputs. In addition to providing insights into living systems, purified actomyosin active gels could be leveraged as actuators in bioinspired microrobots or synthetic cells [37,39]. Indeed, the actomyosin cytoskeleton in living cells has many advantages compared to most actuators. Our results help to shift the framing of inquiry towards thinking of actomyosin's mechanical role in cellular signal and information processing. Future work can begin to propose inquiries about the nature of feedback in these gels. In the way that a proportional-integral-derivative controller regulates the throttle of an automobile to maintain a desired velocity, we ask whether there might be ways to regulate the chemical free energy available to our active gels to achieve certain physical responses?

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J.A. conceptualized the work; J.C., F.C., and A.D. conducted the experiments. J.C., F.C., A.M., and J.A. contributed to the modeling results. J.C. and J.A. led writing efforts.

The authors report no conflicts of interest.

DATA AVAILABILITY

Data for this article are available at the Texas Data Repository at [94].

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