ATP Controls the Mode of Actomyosin Remodelling

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Since myosin-II motors exert forces on actin filaments upon hydrolysis of Adenosine Triphosphate (ATP), the rate at which energy is converted into mechanical work—the power—is directly proportional to the concentration of available ATP. Here we report evidence that changes in power also alter the mode by which mechanical work is carried out by actomyosin. Specifically, as ATP concentration increases, contractility gives way to increasingly processive motion of myosin-II minifilaments along actin filaments, resulting in meta-stable vortex- and spiral-like motifs. This implies a non-trivial coupling between ATP synthesis (via cellular metabolism) and cortical remodelling, with potentially wide-ranging consequences.

Actomyosin, the canonical mixture of actin filaments and myosin-II motors, is crucial for many cellular functions [1–3]. It is also a central example of active matter [4], since the hydrolysis of myosin-II head-groups by Adenosine Triphosphate (ATP) permits a so-called powerstroke, locally converting chemical energy into mechanical forces.

As a result, the rate at which actomyosin does work—i.e., the power—is directly proportional to the concentration of ATP. We now report evidence that changes to levels of available ATP, and hence the power, also change the mode by which mechanical work is done. The implication is that ATP synthesis and cortical function are intimately linked, with particular ramifications for cells whose metabolism is compromised, such as cardiomyocytes under heart disease, for example [5, 6].

Asters, spirals and vortices revisited—We start with actomyosin reconstituted in vitro on supported lipid bilayers [8–14]: a system previously shown to readily form asters and contractile foci under a variety of conditions [10, 11]. We use interferometric scattering (iSCAT) microscopy [15, 16]—a label-free technique that relies on the interference between reflected and scattered light from nano-objects near an interface. iSCAT was recently used to reveal that minifilaments of muscle myosin-II move along intermediate length actin filaments with a dwell time and a run length that depend on ATP [17]. Now, using a similar system, we report evidence that remodelling of the actin meshwork is also ATP-dependent.

Our central observation is that, whilst aster-like contractile foci can be observed once ATP has been depleted (Vid. 1), at earlier times, when concentrations of ATP are intermediate-to-high, we also observe complex swirling patterns. These are characterised by recurrent dynamical vortex- and spiral-like motifs, where myosin-II minifilaments tightly circle either a void or a myosin-II rich centre (Fig. 1d, Vid. 2-5, SM Sec. 2 [18]). On rare occasions, we also see such structures merge to form a larger foci (Vid. 6). The average diameter of the circular motion was 4.5 ± 1.3 μm, with particle image velocimetry (PIV) [19–21] characterising peak speeds on the order of 100 nm s⁻¹ (see SM Sec. 2 [18]). The lifetimes of the motifs all exceeded 90 s, which is far greater than the average residence time of individual minifilaments [17], and indicative of a quasi-stable state whereby the incoming and outgoing fluxes of minifilaments are almost balanced.

To support these findings, we separately observed fluorescently labelled actin filaments under similar conditions using total internal reflection fluorescence (TIRF) microscopy (Fig. 1c and SM Sec. 1 [18]). Again, this revealed long-lived vortex-like motion (Fig. 1e, Vid. 7-8, SM Sec. 2), with circular motifs of average diameter 3.7 ± 1.5 μm and lifetimes > 100 s. Strikingly, however, PIV revealed that speeds were 1–5 nm s⁻¹, significantly slower than those of the myosin-II minifilaments (see SM Sec. 2 [18]). Moreover, the actin and myosin vortices flow in the same direction, and almost all the vortices are of the same handedness. The latter we attribute to the chirality of actin inducing a twist-bend coupling [22–24].

Nominally, such observations bared superficial similarities to the titular structures of the pioneering theoretical study [25]. However, the underlying physics is quite different: with no indications of a spontaneous splay instability, the only true steady-states in our system should, in principle, be aster-like. In order to explain the observed meta-stable structures, therefore, we use a simple model of myosin-II minifilament kinetics to argue that active coefficients in bulk hydrodynamic descriptions must scale non-trivially with the concentration of available ATP.

Minifilament kinetics—At its core, our argument is very simple, and revolves around the fact that myosin-II minifilaments comprise up to ~50 individual motors. The key point is that, the mode by which a single motor’s powerstroke transmits forces to the actin meshwork depends crucially on the status (either bound or unbound) of the other head-groups in the same minifilament. Too few bound head-groups, and the myosin-II minifilaments can essentially walk along individual strands of actin. Too many bound head-groups, however, and any motion is likely inhibited, with forces instead transmitted across
multiple actin filaments.

Consider the kinetics of a single myosin-II minifilament with $N$ identical heads, each of which binds (to actin) with a rate $k_{on}$ and unbinds with a rate $k_{off} \propto [ATP]$ (Fig. 2a-b). Calculating mean first passage time (MFPT) to filament dissociation (SM Sec. 3 [18] & [26]) we see that, the greater the concentration of ATP (i.e., $k_{off}/k_{on}$) the shorter the dwell time, which agrees qualitatively with experiment [17]. For appreciable levels of ATP, the MFPT scales like $k_{on}/k_{off}$ irrespective of $N$ (Fig. 2c), which is qualitatively in line with more complex models of myosin-II minifilaments in the limit of low load, although certain subtleties are overlooked in favor of analytical tractability [27, 28]. Crucially, the minifilament dissociation rate in this regime is dictated almost entirely by the head-group kinetics, and hence ATP.

Since every transition between states of decreasing number (i.e., $i \to i-1$) involves a single powerstroke—i.e., when myosin minifilaments take a step along actin. For all other transitions (i.e., $i \to i-1$ for $i \neq 1, 2$) we assume that the bound head-groups crosslink more than one actin filament, and therefore the powerstroke results in relative motion of two or more filaments. At the scale of bulk hydrodynamic descriptions, this manifests as predominantly contractile forces, since the response of actin filaments is asymmetric when subjected to either compressive or tensile loads [29–32], and the dwell time of myosin-II head-groups at the plus-end of actin filaments is abnormally long [33].

In this context, a proxy for the power associated with a single minifilament’s processivity is the mean number of $2 \to 1$ transitions divided by the mean residence time, which rapidly converges to a constant for increasing ATP (Fig. 2d). Similarly, summing over the mean number of all non-processive descending transitions (SM Sec. 3 [18]) and dividing by the total dwell time, we arrive at a proxy for the power associated with the contractility generated by a single minifilament, which scales linearly with $k_{on}/k_{off}$, and hence inversely with ATP concentration (Fig. 2d).

**Scaling of kinetic coefficients**—To understand whether such behaviour is consistent with our observations, we write down a hydrodynamic theory for our *in vitro* system (SM Sec. 4 [18] & [4, 25, 34–36]). Using the single minifilament run length and dwell time as characteristic spatio-temporal scales, the dimensionless theory is as follows. There are two continuity equations, one for the density of myosin-II minifilaments

$$\partial_t \rho_m + \nabla \cdot \left( \vec{v}_m \rho_m \right) = -k - \rho_m \chi m,$$

and one for the density of actin filaments

$$\partial_t \rho_a + \nabla \cdot \left( \vec{v}_a \rho_a \right) = 0.$$  

The source/sink terms in the myosin-II equation correspond to a constant minifilament on-rate and a density-dependent off-rate, with $k$ the ‘bare’ ratio of these two quantities under $\rho_m \to 0$. These two expressions are coupled by force balance equations at the actin-minifilament and actin-bilayer interfaces. In our layered system (Fig. 1a) these are given by:

$$\vec{v}_m = \vec{v}_a - \vec{F} - \frac{\chi m}{\rho_m} \nabla \rho_m,$$

and

$$\vec{v}_a = \frac{1}{\rho_a} \nabla \cdot \Sigma - \frac{\chi m}{\rho_a} \nabla \rho_m.$$  

**FIG. 1. Meta-stable vortices and spirals.** a) Myosin-II minifilaments were deposited on a layer of F-actin, itself attached with His10-KCK-EzrinABD to a supported lipid bilayer on a glass substrate (not to scale). b) Networks with intermediate length F-actin ($l_{actin} = 8 \pm 4.7 \mu m$ [7]) and an approximate minifilament-to-actin ratio of 3:1 ([actin] = 300 nM, [myosin II] = 80 nM) displayed meta-stable spiral-like structures in iSCAT with an average diameter of 4.5 ± 1.3 µm. c) Observing a range of lengths ($l_{actin} = 9 \pm 5.5/7 \pm 4.7/6 \pm 4.6 \mu m$) of fluorescently-labelled actin via TIRF at an approximate minifilament-to-actin ratio of 1:1 ([actin] = 250 nM, [myosin-II] = 10/20/20 nM) resulted in motifs with an average diameter 3.7 ± 1.5 µm. d) Representative minimal intensity projections (duration 120s, scale bar 2 µm) and corresponding flow fields inferred using PTV analysis for iSCAT. e) Representative vortex TIRF maximal intensity projection (duration 120s, scale bar 2 µm) with corresponding PIV analysis.
FIG. 2. Myosin-II minifilament kinetics. a) The statistics of the bound status of a single myosin-II minifilament’s headgroups can be approximated by a ladderlike state space. b) The powerstroke associated with the transition between doubly- and singly-bound head-groups leads to processive ‘walking’, whereas increased numbers of bound head-groups leads to crosslinking and hence contractility (not to scale). c) As \( k_{\text{off}} \propto [\text{ATP}] \) increases, the MFPT until minifilament dissociation converges to \( \sim 1/k_{\text{off}} \), irrespective of the total number of head-groups. d) Transition rates in the processive (2 \( \to \) 1) and contractile (\( i \to i-1 \) for \( i \neq 1, 2 \)) regimes scale as a constant and \( \sim 1/k_{\text{off}} \), respectively, as \( k_{\text{off}} \propto [\text{ATP}] \) increases.

respectively. Here, \( \chi_m \) is a dimensionless isothermal compressibility for myosin-II minifilaments, \( \vec{P} \) is a polar order parameter, and the tensor \( \Sigma \) is given by

\[
\Sigma = \left( -\zeta + \nu \vec{P} \cdot \vec{h} \right) \mathbb{I} + \nu \left[ \tilde{\rho} \vec{h} \right]^S + \left[ \tilde{\rho} \vec{h} \right]^A + \left( \chi_m \rho_m - \chi_a \rho_a \right) \mathbb{I} + 2 \eta \left[ \nabla \vec{v}_a \right]^A + \bar{\eta} \text{Tr} \left\{ \nabla \vec{v}_a \right\} \mathbb{I}. \tag{5}\]

Sans-serif superscripts \( S \) and \( A \) denote the symmetric and anti-symmetric parts, respectively, whilst \( \zeta \) is the active isotropic contractility. The constants \( \nu \) and \( \bar{\nu} \) are the spin connection coefficients, \( \chi_a \) is the dimensionless compressibility for actin, and \( \eta \) and \( \bar{\eta} \) are the shear and bulk viscosities, respectively. The quantity \( \vec{h} = -\delta F/\delta \vec{P} \), is the so-called molecular field, which is derived from the elastic free energy of the polarisation field. Ostensibly, the low densities involved in our experiments imply that \( F \) can be neglected. However, intermediate length actin filaments have a persistence length that is a non-vanishing fraction of their length, permitting bending at relatively low energetic cost [24]. We approximate this behaviour by including a free-energy of bending, \( \rho \),

\[
F = \int \frac{k}{2} \left\{ \vec{P} \cdot \nabla \vec{P} \right\}^2 \mathbb{d}A, \tag{6}\]

where \( k \) — the bend elastic modulus [37] — is small. The system of equations is then closed by the dynamics of the polar order parameter, which is given by

\[
D_\gamma \vec{P} = \frac{\vec{h}}{\gamma} - \nu \left[ \nabla \vec{v}_a \right]^A \cdot \vec{P} - \bar{\nu} \text{Tr} \left\{ \nabla \vec{v}_a \right\} \vec{P}, \tag{7}\]

where \( \gamma \) controls the relaxation of \( \vec{P} \), and \( \vec{D}_i \vec{P} = \partial_i \vec{P} + \vec{v}_a \cdot \nabla \vec{P} + \left[ \nabla \vec{v}_a \right]^A \cdot \vec{P} \), is the objective rate.

ATP-dependence enters explicitly in (1), since our microscopic model relates the kinetics of entire minifilaments to those of its head-groups, implying \( k \sim k_{\text{on}}/k_{\text{off}} \), which is inversely proportional to [ATP]. It also enters via the active isotropic contractile term, \( \zeta \), where we note that active anisotropic (\( \vec{P}\vec{P} \)-like) terms are omitted based on the observed meta-stable motifs (SM Sec. 4 [18]). We assume that \( \zeta \) has the saturating form

\[
\zeta = \xi \frac{k \rho_m}{k \rho_m + \rho_a}, \tag{8}\]

where, at low ratios of myosin-II minifilaments to actin, the active contractile stress is linear in \( \epsilon = k \rho_m/\rho_a \), and therefore consistent with our simple kinetic model. Indeed, taking \( k \) as a proxy for generic cross-linking between myosin and actin leads us to assume that the dissipative kinetic coefficients \( \eta, \bar{\eta}, \nu, \bar{\nu}, \chi_a \) and \( 1/\gamma \) are all \( O(\epsilon) \) in the low minifilament density regime.

Crucially, the only term that is independent of \( k \) in this dimensionless presentation, is the mono-polar-like processive force \( \vec{P} \) in (3), which is of constant magni-
FIG. 3. Comparing theory and experiment. a) iSCAT microscopy image of an aster where dark areas indicate high myosin-II minifilament density (scale bar 2 µm). Radial steady-state solutions for b) myosin density, c) actin density, and d) the myosin velocity (texture shown in b inset). e) Minimal intensity projection (over 120s) of a spiral-like structure using iSCAT (scale bar 2 µm). f) Radial myosin density and g) velocity profiles at zeroth order in ε (texture shown in f inset). h) Radial actin velocity profile at O(ε).

Comparison with experiment—By inspection, an axisymmetric steady-state solution to Eqs. (2) and (7) is just a radial actin texture that is stationary (i.e., \( \vec{P} = \vec{e}_r \) and \( \vec{v}_a = 0 \)). This implies that \( \vec{v}_m \) is a function of \( \rho_m \), such that the accumulation of myosin-II minifilaments due to their procession towards the texture’s centre is balanced by the density dependent dissociation rate (see Vid. 9 and SM Sec. 2 for this behaviour in experiment). Solving this boundary value problem for \( \rho_m \) requires a shooting method. The solution can nevertheless be substituted into the remaining force balance condition, which equates contractile forces with those resisting the compression of actin. This gives a first order equation for \( \rho_a \) that is readily solvable numerically. The resulting aster-like steady-state is depicted in Fig. 3b. Since it is qualitatively independent of \( k \), we expect that the system converges to this state as ATP depletes. By contrast, in order to explain the long-lived vortex / spiral motifs of our experiments, we must take a perturbative approach: expanding in small \( \epsilon \) (high ATP) and solving hierarchically.

At zeroth order, \( \vec{v}_a^{(0)} = 0 \), implying freedom to impose \( \vec{P}^{(0)} \) and \( \rho_a^{(0)} \). To mimic experimental motifs, we choose a spiral texture

\[
\vec{P}^{(0)} = \frac{r \left( R^2 - r^2 \right)^2 \vec{e}_r + \frac{a^2 r^5}{(r+R)^2} \vec{e}_\theta}{\sqrt{\left( \frac{a^2 r^5}{(r+R)^2} \right)^2 + r^2 \left( R^2 - r^2 \right)^4}},
\]

with density profile \( \rho_a^{(0)} = c r^2 e^{-r^2/w} + l \) (SM Sec. 4 [18]). In this case, the minifilament velocity profile, \( \vec{v}_m \), is that which balances propulsive forces with density gradients. Substituting into (1), the resulting boundary value problem is again solvable via a shooting method. The results are in-line with experimental observations, with an inward flux of minifilaments giving way to a circular motion around a central foci, at which point the high densities are balanced by high dissociation rates.

As might be expected, this state is unstable at first order in \( \epsilon \). We can, nevertheless, calculate first order corrections to the actin velocity profile, \( \vec{v}_a^{(1)} \). In addition to radial corrections, this results in a small circular flow in the same direction as the myosin-II minifilaments (a phenomenon also seen in experiments, Fig. 1e & SM Sec. 2 [18]) that is accompanied by a slight shear-banding. The former is consistent with our observations, whilst the latter is either not present, or cannot be observed at the present resolution.

Discussion—When taken together, experiments, single minifilament kinetics, and bulk hydrodynamics are all consistent with the notion that an increase in ATP not only increases the power of actomyosin, but it also
changes the mode by which work is done. Specifically, we argue that contractile forces give way to more processivity of myosin-II minifilaments at higher levels of available ATP. In other words, the concentration of ATP interpolates between active forces that are Galilean invariant and those that are not. At a hydrodynamic level, this results in a model of actomyosin that has predominantly ‘wet’ characteristics at low ATP, and ‘dry’ characteristics at high ATP.

We speculate that this is not only important for the generic understanding of actomyosin, but also for cells whose metabolism is compromised. The latter is a hallmark of a number of important diseases, including heart disease, where the function of cardiomyocytes is intimately linked to their ability to contract [5, 6]. The ideas put forward here may be important to understanding the details of how such diseases manifest at the scale of bulk actomyosin and therefore impact cellular mechanics.

Nevertheless, there remain aspects of our theory that might be improved upon. Chief amongst these is that state-of-the-art liquid crystal-like hydrodynamic descriptions are insufficient to fully describe the remodelling of networks of intermediate-to-long actin filaments, which are semi-flexible and curved on length-scales similar to the filaments themselves. Whilst such networks have been explored extensively in numerical simulations—particularly in terms of rigidification transitions and contractility generation [29, 38–47]— writing down a constitutive relation for such materials is a significant outstanding challenge in the field. To our knowledge, it has only been attempted at the level of bulk passive rheology thusfar, and has no straightforward generalisation to active systems [48–50]. Amongst other things, such a constitutive relation would likely need to account for the known shear-stiffening of actomyosin under imposed strain [29]. The Poisson bracket approach for computing reactive couplings in generalised hydrodynamics might prove useful here, as in nematic polymer theory [51, 52].

In conclusion, we believe that our work highlights an important conceptual advance. However, there is still much work to be done to understand the rheological details of a full active hydrodynamic description of actomyosin. Developing more precise experimental setups (e.g., microfluidic control of ATP concentrations) and more realistic microscopic theories to feed into coarse-grained hydrodynamic parameters are just two of the possible future directions one could take. We therefore welcome further work in this area.

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