Ciliary oscillations without external friction near the instability threshold

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Active filaments are prototypical engines of motility at the micron scale. Their motion in viscous fluids is determined by the balance of external drag and internal structural stress. The latter contains both active and passive components that, respectively, excite and relax motion. Knowledge of the passive internal and external stresses enables determination of the nature of the active component, which is a key step in the validation of mechanochemical models of activity. Here, we simultaneously measure filament motion and external flow of an isolated and active Chlamydomonas cilium with high spatio-temporal resolution to unambiguously estimate the passive stresses. The measured flow field provides a self-consistent validation of the external drag force, which is found to be local and, surprisingly, negligibly small compared to internal elastic stresses. Consequently, there must exist other sources of friction within filament interior for it to exhibit stable driven oscillations. Incorporating internal dissipation as the sole source of friction and a generic spring-dashpot form of active stress, we show that an instability to oscillations takes place when active stresses are strain-softening and shear-thinning. This is in striking contrast with the conventional wisdom that external fluid friction plays an indispensable role in ciliary oscillations.

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Active filaments are out-of-equilibrium systems that undergo spontaneous oscillations by converting chemical or other forms of energy into mechanical motion [1]. Compared to their equilibrium counterparts, they show novel features in symmetry breaking [2, 3], collective behavior [4, 5] and topological defect dynamics [6–8]. They are usually suspended in a viscous fluid and are ubiquitous in both biological [9–11] and synthetic systems [12–14] for setting up fluid flow and transport. While biofilaments like cilia exhibit periodic bending motion due to internal moment generating molecular motors at the nanoscale, synthetic filaments are driven by electromagnetic torques or phoretic fields which generate slip velocities on the particle surface [3, 13, 15, 16].

The motion of an active slender body in a fluid is governed by the balance of active and passive stresses. The passive stresses contain internal contributions from the structure of the filament and external contributions from fluid friction [17]. Since filament dynamics conserves both momentum and angular momentum, a knowledge of the passive components of the stress allows an estimation of the active component. Eukaryotic cillum is a well-studied system where such balance equations have been extensively used to elucidate the mechanism of oscillations [10, 18–24]. While the internal passive stresses are calculated from its measured waveform, the external viscous drag is generally computed using slender body integral equations, but not validated experimentally. A number of studies have measured both waveform and flow fields with multiple or single cilium attached on live cells [25, 26]. The complex boundary conditions posed by the large cell body and multiple cilia prevents unambiguous measurement of external viscous drag of a single cilium. In addition, live cells have the added complexity of biochemical control of ciliary beating. Here, we present the first simultaneous measurement of the bending waveform and flow field of an isolated, active Chlamydomonas cilium to determine the macroscopic stresses resulting in its periodic bending motion. In addition to the structural response, the measured flow field provides the first self-consistent estimate of the hydrodynamic force acting on the filament. This information is extremely crucial, as will be shown below, to accurately estimate the structural stresses.

Demembranated cilium i.e. the axoneme (10–12 µm in length, 200 nm in diameter) consists of 9+2 microtubule (MT) doublets connected to each other through elastic nexin links and radial spokes [27, 28]. In addition to these passive components, the active dynein motors, anchored on one of the MTs of each doublet, consume adenosine triphosphate (ATP) to slide the MTs with respect to one another. However, inter-MT sliding is restricted through the elastic connections and consequently the sliding motion is converted into bending [29]. The axoneme transitions from an initially stable quiescent state to an oscillatory state at a critical ATP concentration through an instability threshold [30]. The intricate details of the internal structure of an axoneme become irrelevant near this threshold, where nonlinear contributions are small. Therefore, we perform experiments near critical ATP concentration and model the axoneme as a shearable active rod where we eliminate all the details related to its internal structure and calculate stresses averaged over its cross-section.

Our principal results are as follows: The external hydrodynamic drag for a Chlamydomonas axoneme is essentially local, with negligible contribution due to long-range hydrodynamic interactions. Surprisingly, the external drag is negligible compared to the elastic forces within the filament. This implies that internal friction within the filament itself is the primary source of dissipation. We consider a simple model for an active filament that includes a generalized spring-dashpot form for the active stresses and dissipation only through internal friction. At the level of a linear analysis of this model, relevant in context of our experiment operating near the critical ATP concentration, we show that ciliary oscillations exist when active stresses are strain-softening and shear-thinning. These results challenge the common view of external fluid friction being essential in ciliary oscillations. In the regime of experimentally relevant parameters, the viscous shear-thinning component of the active response dominates over the elastic strain-softening one.

**EXPERIMENTAL SYSTEM**

The experimental system consists of isolated axonemes, purified from the unicellular algae Chlamydomonas reinhardtii, and reactivated in-vitro by the addition of ATP (Methods, SI). The axonemes are 11 µm in length and clamped at one end on a glass cover slip, whose oscillations are approximately planar with an average centreline height of ∼ 0.9 µm from the surface. Passive microspheres are introduced into the suspension as tracers for measuring flow of the ambient fluid using particle tracking velocimetry (PTV) (Methods). We capture motion of both the axoneme and tracers at high spatio-temporal resolution using a 60X phase objective coupled with a high speed camera (1200 frames per second). Figure 1a shows time lapse images of the axoneme and tracers at selected time points through the beat cycle (Movie S1).

The position of the axoneme is sampled at sufficiently many points over its length to construct a global Chebyshev
polynomial interpolant of the parametric form \( R(s) = \sum a_n(t)T_n(s) \) where \( 0 \leq s \leq L \) is the arc-length and \( a_n \) is a vector of Chebyshev coefficients for the \( x \) and \( y \) components of the position \([31]\). The discretely sampled axoneme and the interpolated centreline are shown in Fig. 1b. Higher order derivatives of the interpolation lose accuracy at the base and terminus of the axoneme and data from those parts are, accordingly, discarded (SI, Fig. S1).

FILAMENT GEOMETRY AND MECHANICS

We model the active filament as an inextensible, but shearable, rod of uniform circular cross section of diameter \( a \) and length \( L \), with a centreline described by the curve \( R(s) \). To each point of the curve is attached an orthogonal Frenet-Serret frame whose rate of rotation is described by the Darboux vector \( \Omega(s) \). Then, the spatial rate of change of the curve tangent, \( t \), is \( \partial_s t = \Omega \times t \) with similar equations for the normal \( n \) and the binormal \( b = t \times n \). The Darboux vector is related to these by \( \Omega = \tau t + \kappa b \), where \( \tau \) and \( \kappa \) are, respectively, the curvature and torsion of the curve. We restrict ourselves to motions with vanishing torsion for which the Darboux vector is normal to plane of motion and the Frenet-Serret frame is aligned with the tangent and the normal, resulting in \( \Omega = \partial_s \theta b \), where \( \theta(s) \) is an angle. The tangent automatically satisfies the constraint of inextensibility \( t \cdot t = 1 \) when parametrised by this angle as \( t(s) = [\cos \theta(s), \sin \theta(s)] \). The position of the curve is obtained in terms of the tangent angle by integrating \( \partial_s R = t \). The shear strain of the rod parallel to its centreline is \( u(s) \) and it is assumed that the strain vanishes at the clamped base, \( u(0) = 0 \). Inextensibility then requires

\[
u(s) = a[\theta(s) - \theta(0)] \equiv a\Delta \theta(s)
\] (1)

and the kinematics of the filament is completely specified by the tangent angle \( \theta(s) \) or equivalently by shear angle \( \Delta \theta \). Notably, the unobservable shearing of the filament can be inferred from its observable curvature, since \( \kappa = \partial_s \theta \equiv \partial_s \Delta \theta \).

There is no assumption of small curvature in this kinematic description. Internal active stresses cause the filament to shear and, by the kinematic constraint, to curve.

We assume that the rod supports an internal stress whose stress and moment resultants \([32]\) on the cross section at \( s \) are, respectively, \( F(s) \) and \( M(s) \) and that it is acted upon by forces and moments whose sum per unit length are, respectively, \( f \) and \( m \). Then, in the absence of inertia, the balance equations for force and torque are \([33, 34]\)

\[
\partial_s F + f = 0, \quad \partial_s M + t \times F + m = 0
\] (2)

Internal moments included in \( m \), which can exist only if the internal stress is antisymmetric, are generally omitted in the elasticity of rods, as for example in \([35]\), but are essential to our model. The above equations are closed by identifying the relevant forces and moments and then providing their constitutive equations in terms of the kinematic variables. Integrating the force equation, it follows that the stress resultant to be used in the torque equation can be expressed as \( F(s) = \int_0^s f(s')ds' + F(L) \), where \( F(L) \) is specified by the boundary condition at the terminus of the rod. At a free terminus, the stress resultant vanishes giving \( F(L) = 0 \). The only force per unit length relevant here is the viscous drag, \( f^v \), resulting from the motion of the rod relative to the ambient fluid.

EXTERNAL VISCOUS DRAG

A rod moving through a viscous fluid experiences a drag \( f^v(s) \) and creates a flow \( v(r) \). These are related through the conservation of fluid momentum. In the experimentally relevant limit of slow viscous flow and a slender rod, \( L \gg a \), the integral representation of Stokes equation gives \([36]\)

\[
v(r) = -\int_0^L G(r, R(s)) \cdot f^v(s) ds.
\] (3)

This represents a distribution of Stokeslets of strength \( f^v(s) \) where \( G \) is a Green’s function of the Stokes equation. The matching of the fluid flow to the velocity of the rod at its surface yields the slender body integral equation whose formal solution is \( f^v(s) = -\int_0^L \gamma(s, s') \cdot \dot{R}(s') ds' \). This relates the drag on any part of the rod to the velocity of its centreline. The non-local friction kernel \( \gamma(s, s') \) can be obtained to any desired accuracy by numerical methods \([37]\). It is often assumed that the friction kernel can be approximated by a local form with a constant friction coefficient, \( \gamma(s, s') = \gamma \delta(s - s') \). In this resistive force theory (RFT) limit, the drag is \([38]\)

\[
f^v(s) = -\gamma \cdot \dot{R}(s), \quad \gamma = \gamma_n nn + \gamma_t tt
\] (4)
with the coefficient of normal friction $\gamma_n$ being approximately twice that of the tangential friction $\gamma_t$. Here $\gamma$ is a second rank tensor, while $\mathbf{\bar{R}}(s) = \mathbf{\bar{R}}_n(s)\mathbf{n} + \mathbf{\bar{R}}_t(s)\mathbf{t}$ is the velocity vector. 

With the drag thus determined in terms of the velocity of the centreline, we now validate the RFT form of the viscous drag using the experimentally measured instantaneous flow fields (Fig. 2a,b). The direction of the streamlines vary during the cycle, as is expected from distinct power (Fig. 2a) and recovery (Fig. 2b) stroke waveform. We compare these experimental flow fields with theoretically computed ones using Eq. (3) and (4) (Fig. 2c,d), wherein the filament centreline velocity is determined from the measured waveform and both drag coefficients and Green’s function for flow near a no-slip wall are used from the literature (SI). Representative cuts along the experimental and theoretical flows show that there is a good agreement between the two (Fig. 2e,f). A more comprehensive comparison is given by the root mean square deviation of the flows which is nearly in the Brownian noise regime implying a good match (SI). Therefore, we have now verified by direct measurement the validity of the local relation between filament velocity and force and determined the drag force unambiguously. Figure 2g,h show the variation of the normal and tangential components of the external drag force density over three beat cycles. This form of the drag is used to evaluate the stress resultant and its normal component is used in the torque balance equation below.

**SCALAR EQUATION OF MOTION OF THE FILAMENT**

In slow viscous flow as is shown above, the drag acts in the plane of motion and the stress resultant remains in that plane. For vanishing torsion, the couple resultant is normal to the plane of motion, $M = Mb$, and vanishes at a free terminus, giving $M(L) = 0$. As the only non-zero components of $\mathbf{t} \times \mathbf{F} = \mathbf{F}_n \mathbf{b}$ and $\mathbf{m}$ are normal to the plane, torque balance reduces to a scalar equation for $M$.

$$\partial_s M + F_n + m = 0$$

(5)

The dissipation in the above equation is contained in the normal component of the stress resultant due to external viscous drag as $F_n(s) = -\gamma_n g_n(s)$ (SI), which is determined from the experimentally verified RFT form of drag. The simplest elastic contributions for an inextensible and shearable active rod are that due to bending, $M = EI\kappa$, and shear, $m = -aku$ [18, 35, 39]. The source of filament motion is included as the active moment per unit length, $m^4$. Material parameters in these constitutive relations are the Young’s modulus, $E$, the moment of inertia, $I$ and the shear stiffness, $k$. Torque balance, closed by the constitutive relations and the expression for the stress resultant, yields the following dynamical equation for the curvature of the rod:

$$EI\partial_s \kappa - aku - \gamma_n g_n + m^4 = 0$$

(6)

All the passive terms in this equation can be expressed completely in terms of the tangent angle ($\kappa = \partial_s \theta \equiv \partial_s \Delta \theta$, Eq. (1), SI).

A closer examination of the experimental data reveals that the tangent angle can be more intuitively parametrised by a travelling wave form

$$\theta(s,t) = \theta_0(s) \sin[\omega t - \phi(s)] + \bar{\theta}(s)$$

(7)

where $\theta_0(s)$ is the amplitude of the travelling wave, $\omega$ is the angular frequency of the beat, $\phi(s)$ is the phase and $\bar{\theta}(s)$ is an offset [23, 40]. We compare the experimental data with this parametric form in Fig. 3 and find an excellent fit. As we are interested in oscillations about the time averaged shape of the beat ($\bar{\theta}(s)$), we define $\theta'(s,t) = \theta(s,t) - \bar{\theta}(s)$. In the following, we focus on $\theta'$ and drop the prime such that $\theta \equiv \theta_0(s) \sin[\omega t - \phi(s)]$ which represents the dynamic oscillatory beat of the filament about the mean shape. Therefore, we use this parametric form, instead of the Chebyshev interpolant, to estimate all angle-dependent quantities in the dynamical equation.

We identify $l_\kappa = \sqrt{EI/a^2k}$ as the curvature penetration length scale and $v_n = EI/\gamma_n l_\kappa^2$ as the hydrodynamic relaxation frequency scale of the system through the dynamical equation (6). Measuring $s$ in units of $l_\kappa$ and $t$ in units of $1/v_n$, the dimensionless equation of motion is

$$\partial_s^2 \Delta \theta - \Delta \theta - g_n + m^4 = 0$$

(8)

where $m^4$ is rescaled by $l_\kappa^2/EI$. With the elastic and drag parameters from the literature (SI) and shear angle from the parametrised travelling waveform, the space-time variation of elastic and viscous terms of the above equation are plotted in color scale in Fig. 4a. While the elastic terms are linear in the shear angle and have travelling wave nature, the normal component of stress resultant due to viscous drag, being nonlinear, has in addition a major standing wave component [19, 40]. On comparison of the colorbars in Fig. 4a, we conclude that the hydrodynamic dissipation (panel
III) is negligible compared to the elastic forces (panel I & II). This can also be inferred from the fact that \( \nu_h \gg \nu_b \), \( \nu_b \) being the beat frequency of the filament (SI).

The axoneme being a driven oscillator, stability requires a source of dissipation that is not negligibly small. Since external fluid friction cannot account for this, consistency demands that the internal stress have, in addition to an elastic component, a dissipative frictional component. Each kinematic degree of freedom, i.e., bending and sliding, can contribute to dissipation. Bending friction in MT has been experimentally demonstrated to be significant for length scales smaller than 20 \( \mu \)m and attributed to slow internal structural rearrangements [41, 42]. The bending friction coefficient of an axoneme, \( \Gamma_n = 1.6 \) pN\( \mu \)m\(^2\)/s, is same as that of a single MT since it is an intensive quantity [42, 43]. Though there is no experimental measure of the shear friction coefficient of an axoneme, several experimental studies suggest the presence of inter-MT sliding friction [44, 45]. We consider the shear friction coefficient of an axoneme to be \( \Gamma_u = 10 \) pNs/\( \mu \)m from estimates of nexin protein friction [46] (SI). Earlier work introduced similar terms for internal viscous stresses for either stabilization of the numerical simulations of bend initiation and propagation [20, 47–49] or on the basis of theory alone [22, 50]. However, here we show, experimentally, that such terms are necessary to completely account for dissipation in ciliary motion.

**DYNAMICAL EQUATION WITHOUT EXTERNAL FRICTION**

We, therefore, neglect the external viscous drag and include the internal viscous stresses to re-write the scalar form of torque balance equation as \( \partial_t M + m = 0 \). Hence, the constitutive equations are modified as \( M = EI\kappa + \Gamma_n\kappa - m = -ak\nu - \Gamma_u\dot{\nu} + m^A \). In terms of the dynamical variable \( \Delta \theta \), the modified dynamical equation for an axoneme is

\[
EI(\partial_s^2 \Delta \theta + \Gamma_n \partial_s^2 \partial_t \Delta \theta - a^2 k \Delta \theta - a \Gamma u \partial_t \Delta \theta + m^A = 0)
\]

(9)

The negligible role of fluid friction leads to a second-order reaction-diffusion equation for the shear angle, rather than fourth-order partial differential equations that are commonly obtained when fluid friction is retained [21, 22, 49]. Identifying the two frequency scales of the system, a curvature relaxation frequency scale, \( \nu_u = EI/\Gamma_n \) and a sliding relaxation frequency scale, \( \nu_u = ak/\Gamma_u \), we note that \( \nu_u/\nu_b = 9.38 \) i.e both kinematical degrees of freedom contribute to dissipation. Measuring \( s \) in units of \( l_n \) and \( t \) in units of \( 1/\nu_u \), the dimensionless equation of motion is then

\[
\partial_s^2 \Delta \theta + \partial_s^2 \partial_t \Delta \theta - \Delta \theta - \frac{\nu_u}{\nu_u} \partial_t \Delta \theta + m^A = 0
\]

(10)

where \( m^A \) is rescaled by \( l_n^2/EI \). Figure 4b shows the variation of the internal viscous stresses over three beat cycles along the filament length. Their colorbars indicate that they are of the same order as the elastic stresses in Fig. 4a.I,II and hence in competition with each other, unlike the negligibly small external viscous stress.

The internal passive stresses being completely defined, a constitutive relation for the active moment in terms of kinematic variables is required. We assume a minimal spring-dashpot form for the dynamics of the active moment, \( m^A \), coupled linearly to the shear angle \( \Delta \theta \) as \( \partial_t m^A + b_1 m^A = b_2 \Delta \theta \) [30, 51]. Here, the active stress is parametrised by two constants, where \( b_1 \) controls the autonomous dynamics of the active moment and \( b_2 \) controls the amount of feedback it receives from the sliding kinetics. We have chosen this simplest form of constitutive relation as it is first order in time, lowest order in wavenumber and linear in \( \Delta \theta \). The constitutive relation for active moment together with the dynamical equation for the shear angle (10) form a pair of coupled equations, written in matrix form as

\[
\partial_t \begin{bmatrix}
\frac{(\partial_s^2 - \nu_u/\nu_u) \Delta \theta}{m^A} \\
1 - \frac{\partial_s^2}{b_3} - \frac{1}{b_1}
\end{bmatrix} \begin{bmatrix}
\Delta \theta \\
m^A
\end{bmatrix} = 0
\]

(11)

This equation emphasizes that the passive and active parts in this model are independent, but coupled, degrees of freedom.

The coupled dynamical equations are dissipative linear partial differential equations, which can sustain stable oscillations only in the presence of non-linearities. In the following, we focus on the linear regime and seek to identify the threshold for the onset of the oscillations and the frequency of the oscillations near threshold. This is relevant in the context of our experimental results because the axoneme in this study is beating at 60 \( \mu \)M ATP, near the critical ATP concentration of 45 \( \mu \)M at which oscillations set in (Fig. 4c). Hence, the axoneme is beating near the threshold for the onset of oscillations, also called the instability threshold, where the nonlinearity is weak and the oscillation frequency of the limit cycle is that of the linear analysis evaluated at the threshold [30, 52].

We perform linear stability analysis on the coupled dynamical equations (S12) in the Fourier domain. The dispersion relation is quadratic in complex frequency \( z \) (SI),

\[
[(1 + q_n^2) - iz(q_n^2 + \nu_u/\nu_u)](iz - b_1) + b_3 = 0
\]

(12)
The boundary conditions on both ends of the filament discretizes the wavenumbers to \( q_n = \frac{n\pi}{2(L/l)}; n = 1,3,5... \) (odd) (SI). Figure 4d shows the phase space where the complex conjugate roots \( z \) lead to decaying (grey region) or growing (blue region) oscillations for the fundamental mode. Unstable oscillations are possible only when both \( b_1 < 0 \) and \( b_3 < 0 \). The parameters, \( b_1 \) and \( b_3 \), can alternatively be related to the elastic and viscous response coefficients of the active stress (SI, [21, 30]). The sign of the response coefficients being positive implies that active stresses must be strain-softening and shear-thinning for the system to oscillate (SI). A zoomed in view of Fig. 4d.I near the instability threshold is shown in panel (II). The experimental beat frequency of the axoneme that active stresses must be strain-softening and shear-thinning for the system to oscillate (SI). A zoomed in view of Fig. 4d.I near the instability threshold is shown in panel (II). The experimental beat frequency of the axoneme (\( \omega_{\text{exp}} = 2\pi b_1/\nu_b = 0.272 \)), operating close to the threshold, constrains the parameters of the constitutive relation at hand, namely, \( b_1 = -0.121, b_3 = -0.85 \) for the fundamental oscillatory mode. Elastoviscous response coefficients of the active stress computed with these values of \( b_1 \) and \( b_3 \) imply that the viscous response of the active stress dominates the elastic one in our experiment (SI).

**DISCUSSION**

Our simultaneous measurement of waveform and flow field of a *Chlamydomonas* axoneme at high spatio-temporal resolution demonstrates that hydrodynamic dissipation, accurately described by RFT, is negligible compared to internal elastic stresses. Consequently, a dissipation mechanism internal to the filament is essential for stable ciliary oscillations, in contrast to the widely-held view that fluid friction is the source of the only significant contribution to dissipation [20, 23]. We have combined these insights with a theoretical model of filament motion that includes internal friction and a minimal spring-dashpot form of active stress. There exist critical values of active stress beyond which the model exhibits oscillations, namely, active stresses should be strain-softening and shear-thinning. The model is consistent with our experiment when viscous response of the active stress dominates over the elastic one. On comparing the nature of active stress so obtained with a microscopic model of load dependent detachment of motors [21, 30], we infer that axonemal dyneins are low duty ratio motors (SI).

We mention that though individual aspects of the theoretical model have been considered previously, in particular internal shear and bending friction, non-existent external dissipation and spring-dashpot active sliding stress, to the best of our knowledge, this is the first instance in which all of them have been combined and then situated within the exact theory of stress and strain (SI). The model has been analysed using experimental literature values of all the material parameters of axoneme/MT except for the shear friction coefficient. This uncertainty can be lifted by experimentally measuring the shear friction coefficient for an axoneme possibly by measuring the dynamical stress relaxation to longitudinal step deflections as in [53]. We note that the value of the shear friction coefficient does not affect the existence of oscillation rather it modulates the magnitudes of the viscoelastic response coefficients of the active stress.

The analysis of this model far from the instability threshold, and with the consequent inclusion of non-linear terms, is of considerable interest but outside the scope of the present study. The developed formalism in this article along with the non-linear corrections can be used to connect the microscopic mechano-chemistry of biofilaments to the measured macroscopic active stresses. For example, careful tuning of the experimental parameters that affect the waveform and/or flow field of the motile filament will piece together a comprehensive understanding of the mechanism of their beating. Finally, this approach of simultaneous motion and flow measurements can serve as a paradigm for analysis of active filaments, both biological and synthetic ones, which have wide applications in, amongst others, cargo transport, drug delivery, microfluidics [10, 12–14, 16]. The measured flow field can distinguish internally active filaments from those driven by surface forces (SI, Fig. S3) [54]. In conclusion, therefore, a measurement of the flow field of an oscillating cilium provides vital information on its mechanisms of operation that cannot be obtained from the measurement of motion alone.

**MATERIALS AND METHODS**

**Axoneme purification, sample preparation and imaging**

The unicellular biflagellated alga *Chlamydomonas reinhardtii* (strain: CC1690, wild type), sheds its flagella on long term (~ 5 – 7 hours) exposure to a non-ionic detergent (IGEPAL CA-630) (SI). The plasma membrane covering flagella is disrupted in presence of detergent. The demembranated flagella, when isolated from their cell bodies by centrifugation, are called axonemes. These non-motile axonemes regain their motility in presence of 50 µM - 1 mM ATP (Fig. 4c) [55]. For simultaneous flow field measurements, we add 200 nm sulfate latex beads, coated with long
polymer chains of PLL-PEG20k to impart steric stabilisation (SI). We introduce the reactivation buffer containing axonemes and beads inside a sample chamber made up of a cleaned, polyacrylamide grafted glass slide and coverslip sandwich with double sided tape of thickness 65 µm as the spacer. Prior to sample injection, the coverslip is modified randomly with sticky patches of 0.05% poly-L-lysine over the polyacrylamide brush, to clamp the axonemes at one end (SI). The sample is mounted on an inverted microscope (Olympus IX83), equipped with 60X oil immersion phase objective (0.65-1.25 NA, UPlanFL N) and connected to a high speed CMOS camera (Phantom Miro C110, Vision Research, frame rate ∼ 1200 – 2500 fps) for imaging.

Particle Tracking Velocimetry

The recorded videos are background subtracted, before tracking the tracer displacements, to consider only those tracers which are not stuck to the coverslip and are following the flow. We then perform 2d cross correlations of filament conformations at time $t$ and $t + \Delta t$ over the whole recorded sequence of ∼ 940 beat cycles. The correlation peaks indicate the frames which have similar conformation. The stack of matched conformations are checked manually to delete any conformation with more than 10% dissimilarity. The displacement of tracers in between these two conformations are calculated using standard Matlab tracking routines [56], and velocity vectors are obtained from ∼ 370 – 700 beat cycles. The resulting velocity vectors are placed on a 29 × 29 mesh grid, each of size 0.74 × 0.74 µm over the image, and the mean at each grid point is computed. The gridded velocity vectors are further smoothened using a 2 × 2 point averaging filter.

Data Availability: The data that support the findings of this study are available from the corresponding author on request.

Author Contributions: RA and PS designed the research, DM performed the research and all authors wrote the paper.

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FIG. 1. Simultaneous measurement of motion and flow. (a) Time lapse snapshots of a clamped and reactivated axoneme at 60 μM ATP in presence of 200 nm tracer particles (black dots). The top and bottom panels show the power and recovery stroke of the filament, respectively. Scale bar, 4.2 μm. (b) Non-dimensionalised experimental positions for the first 7 panels of (a) are represented as open circles. The solid lines represent the bivariate Chebyshev interpolant (order 14) to each of the experimental conformations. The clamped end of the filament is set at (0,0).
FIG. 2. External drag force is given by RFT. Experimentally measured instantaneous flow fields using PTV during (a) power stroke with $\Delta t \approx 3.33$ ms time gap between initial and final conformation, (b) recovery stroke with $\Delta t \approx 4.98$ ms time gap between initial and final conformation. The initial conformation of the filament is overlayed on the flow fields in red. Streamlines are plotted for flows with speed greater than $8 \mu$m/s and vector fields for lower speeds in the Brownian noise regime. Scale bar, $3 \mu$m. Theoretically computed flow fields using the slender body approximation and resistive force theory for (c) power stroke corresponding to (a); (d) recovery stroke corresponding to (b). Comparison of experimental (closed circles) and theoretical flow fields (open squares) along representative cuts (dashed lines) for (e) power stroke (f) recovery stroke. The magnitude as well as the range matches well between the two. Space-time plots of the (g) normal $f^v_n$ and (h) tangential $f^v_t$ component of the external viscous drag. The colorbar represents the magnitude of the force density with scaling parameter, $\eta L/T = 0.18$ pN/$\mu$m.
FIG. 3. Traveling wave parametrisation to tangent angle. (a) Space-time plot of the experimentally obtained tangent angle $\theta$ of the filament for three beat cycles. (b) Traveling wave fit (solid lines) to the experimentally obtained $\theta$ (open circles), at representative arc-lengths along the filament. The y-axis does not represent the absolute values of $\theta$. Plot for $\theta$ at each $s$ is shifted along y-axis, for clarity. (c) Space-time plot of the reconstructed $\theta$ from the traveling wave fit, matches well with panel (a). The colorbar to the right of the space-time plots represent their magnitude. (d) The amplitude $\theta_0$ (circles), phase $\phi$ (stars) and shift $\bar{\theta}$ (squares) of the traveling wave in $\theta$ are plotted along arc-length. The solid lines indicate the Chebyshev interpolation of order 10 to each.
Decaying oscillations
Decaying oscillations
Unstable oscillations $b_3 = -0.85$
$\nu_\kappa = -0.121$

**(c)** Phase diagram of the beat frequency as a function of ATP concentration for *Chlamydomonas* axonemes. The axonemes are quiescent below 45 µM ATP (grey). Spontaneous oscillatory beating sets in above 45 µM ATP (unshaded). The blue circles indicate changes in experimental beat frequency of the axonemes with ATP concentration. The experiment in this article is at 60 µM ATP (red star), hence performed near the threshold, where spontaneous oscillation sets in. (d) Oscillatory nature of the complex frequency $z$ from linear stability analysis of the coupled dynamical equations in the parameter space $(b_3, b_1)$ for the fundamental mode $q_1 = \frac{\pi}{2L/l_\kappa}$. The grey and blue shaded regions indicate decaying and unstable oscillations respectively. White patches indicate that there are no oscillatory solutions. (II) is a zoomed in view of (I) near the instability threshold. The experimental beat frequency, scaled by $\nu_\kappa$, $\omega_{\text{expt}} = 0.272$ (black circles) fixes the active model parameters $(b_1 = -0.121, b_3 = -0.85)$ near the instability threshold.
EXPERIMENTAL METHODS

Axoneme purification and reactivation

Wild-type *Chlamydomonas reinhardtii* cells (strain: CC1690) are synchronously grown in 12:12 hours light:dark cycle in TAP+P medium (Tris Acetate Phosphate) [57]. They are collected 2-3 hrs post the beginning of light cycle, at OD$_{750} \approx 0.15 - 0.25$, followed by washing in HES buffer (10 mM HEPES, pH 7.4, 1 mM EGTA, 4% Sucrose) thrice. The plasma membrane covering the cells is disrupted by adding 0.15% IGEPAL CA-630 (I8896, Sigma) in HMDEK buffer (30 mM HEPES, pH 7.4, 5 mM MgSO$_4$, 1 mM DTT, 1 mM EGTA, 50 mM K-acetate) to the cell pellet. The cells in IGEPAL are then kept in ice for $\sim 5 - 7$ hours. These cells are devoid of cell membranes and are called cell models. Some of them shed their demembranated flagella, called axonemes, due to weakening of the attachment to the cell body. Isolated axonemes are then separated from the cell models by centrifugation. Axonemes are then mixed with 30% saturated sucrose, flash freezeed and stored in -80°C for long term usage.

This method of purification, by long term exposure to detergent, yields non-sticky axonemes, in contrast to the commonly used dibucaine procedure [55, 57, 58], hence essential for flow field measurements. These non-motile axonemes regain their motility in presence of 50 $\mu$M - 1 mM ATP in HMDEKP buffer (HMDEK + 1% PEG-20k) and are said to be reactivated. There was no distinction in the nature of beating of the frozen axonemes, when thawed, from those that were not frozen. We use an ATP regeneration system, to hold the ATP concentration constant within the sample for approximately 40 mins, enough for imaging approximately 6-7 isolated axonemes in one sample. The ATP regeneration system comprises of 6 mM sodium creatine phosphate (SCP) [27920, Sigma] and 40 U/ml creatine phosphokinase from bovine heart (CPK) [C7886, Sigma]. The commercially available CPK are typically oxidized, hence we reduce them by DTT at room temperature for efficient reactivation [59].

Tracer particles

The tracer particles chosen, for flow field measurements, are neutrally buoyant (polystyrene microspheres with density 1.055 g/cc) and small (200 nm diameter, the lowest size that can be used with diffraction-limited optical imaging) so that their motion is nearly identical to the fluid in which they reside. Commercially available charge stabilized microspheres stick to each other and to the axoneme due to screening of electrostatic interactions by the divalent ions and salts present in the reactivation buffer. We graft long chains of block co-polymer PLL-PEG20k (PLL, P7890, 15-30 kDa, Sigma; mPEG-SVA-20k, NANOCS), onto 200 nm negatively charged sulfate latex beads (S37491, Thermo Scientific) to impart additional steric stabilisation [60].

Surface modification of slides and coverslips

We clean the coverslips and slides with hot soap solution (1% Hellmanex-III), followed by ethanol and 100 mM potassium hydroxide. We graft polyacrylamide brush on the glass surfaces to suppress depletion interaction of beads and filament with the surfaces. The concentration and timing of the sticky PLL patching is tuned to be such that the reactivated axonemes are clamped at one end for not more than $\sim 1 - 1.5$ $\mu$m.

Depth of focus adjustment and imaging

We use an intermediate numerical aperture (NA) between 0.65 to 1.25 for the 60X variable NA phase (PH3) objective, to capture most of the filament beat in focus. We have measured the depth of focus ($\Delta Z$) of the objective at this intermediate NA to be 1.4 $\mu$m. We only image time lapse sequences of those axonemes which are clean, clamped at one end with proper beating, having 80 – 90% of the filament in focus at a $\Delta Z$ of 1.4 $\mu$m and do not have another reactivated filament in the surrounding area of 30 $\times$ 30 $\mu$m$^2$. The frame rate in the high speed imaging is suitably chosen to capture approximately 1000 beat cycles per axoneme, with $\sim 55 - 75$ conformations per beat cycle, for example 1200 frames per second for movie S1, whose beat frequency is approximately 16.63 Hz.
THEORETICAL METHODS

Hydrodynamics near a no-slip boundary

The Stokes equation for low-Reynolds number flows with the incompressibility condition is
\[ \nabla p(r) - \eta \nabla^2 \mathbf{v}(r) = 0, \quad \nabla \cdot \mathbf{v}(r) = 0 \] (S1)
where, \( p \) is the pressure field, \( \mathbf{v} \) is the fluid velocity. We assume the boundary to be at the \( z = 0 \) plane of the coordinate system, and hence, the filament is at plane \( z = h \). All velocity components must vanish at the no-slip boundary, i.e. \( \mathbf{v}(x, y, z = 0) = 0 \). The Green’s function satisfying this condition is called the Lorentz-Blake tensor \( G_{ij}^B \). The Lorentz-Blake tensor for fluid velocity at field point \( \mathbf{r} \) induced by a point force at \( \mathbf{R} \) is given by the Oseen tensor and the coupling fluid wall tensor, due to the image contribution from the source Stokeslets:
\[ G_{ij}^B(\mathbf{r}, \mathbf{R}) = G_{ij}^O(\mathbf{r'} - \mathbf{R'}) + G_{ij}^D(\mathbf{r''} - \mathbf{R''}) - G_{ij}^{SD}(\mathbf{r''}), \quad (i,j = x, y, z) \] (S2)
where, \( \mathbf{R} = (x, y, z) \) is the Stokeslet position; \( \mathbf{R'} = (x, y, -z) \) is the position of the image Stokeslet due to the wall; \( \mathbf{r'} = \mathbf{r} - \mathbf{R} \), \( \mathbf{r'} = |\mathbf{r'}| \) is the distance between the source Stokeslet and field point; \( \mathbf{r''} = \mathbf{r} - \mathbf{R''} \), \( \mathbf{r''} = |\mathbf{r''}| \) is the distance between the image Stokeslet and field point. The Oseen tensor \( G_{ij}^O \), the potential dipole \( G_{ij}^D \) and the Stokes doublet \( G_{ij}^{SD} \) are well known singularities in fluid flow, whose explicit forms are given by the following [61, 62],
\[ G_{ij}^O(\mathbf{r'}) = \frac{1}{8\pi\eta} \left( \frac{\delta_{ij}}{r^3} + \frac{r_i' r_j'}{r^5} \right), \quad (i,j = x, y, z) \] (S3)
\[ G_{ij}^D(\mathbf{r''}) = \frac{2h^2(1 - 2\delta_{jz})}{8\pi\eta} \left( \frac{\delta_{ij}}{r^3} - \frac{r_i'' r_j''}{r^5} \right) \] (S4)
\[ G_{ij}^{SD}(\mathbf{r''}) = \frac{2h(1 - 2\delta_{jz})}{8\pi\eta} \left( \frac{\delta_{ij} r_j''}{r^3} - \frac{\delta_{ij} r_j''}{r^3} - 3 \frac{r_i'' r_j''}{r^3} \right) \] (S5)
Flow field for each conformation is theoretically computed assuming a line distribution of Stokeslets of strength \( f^v \) along the axoneme centreline \( \mathbf{R}(s) \) (Eq. 3 in main text). For comparison with the experimental flow fields, the theoretical flow is integrated over an infinitesimal time \( \Delta t \), which is the increment between images used for tracking the experimental flow (Methods, PTV). Furthermore, as the experimental depth of focus is 1.4 \( \mu \)m, the theoretical flow is also depth averaged over this \( \Delta Z \).

Root Mean Square Deviation (RMSD) calculation

The match between experimental and theoretical flow fields is quantified by root mean square deviation (RMSD) of the theoretical speed from the experimental one. We calculate RMSD of speed from its residual of the squared deviation (RSD). \( RSD = \sum_{i=1}^{NS}(v_i^{\text{expt}} - v_i^{\text{th}})^2 \), where \( NS \) is the number of grid points, \( v_i^{\text{expt}} \) and \( v_i^{\text{th}} \) are the experimental and theoretical speeds at the \( i \)-th grid point respectively and \( RMSD = \sqrt{ \frac{\text{RSD}}{NS} } \). The RMSD of the theoretical flow from the experimental one in Fig. 2a,c and b,d are 7.47 \( \mu \)m/s and 9.81 \( \mu \)m/s respectively. We have also checked that RMSD for other conformations along the beat cycle is below 10 – 11 \( \mu \)m/s, which is within the Brownian noise regime. The small mismatch between the theoretical and experimental flows is arising from the fact that the filament beat is not completely planar, with its clamped head being at \( z = 0 \) and tail beyond \( z = 1.4 \) \( \mu \)m. The distal end of the filament is mostly out of focus during the beat cycle, as is evident from the 2nd-4th panels in Fig. 1a. Moreover, the filament has a non-uniform beat frequency \( \nu_b = 16.63 \pm 0.62 \) Hz, over \(~ 940\) beat cycles, and the experimental flow field is the average over these beat cycles. But in the theoretical calculation of filament velocity we have considered \( \nu_b = 16.44 \) Hz for the particular beat shown and reconstructed in Fig 1a.
Normal component of stress resultant

The normal component of stress resultant for a filament in a viscous fluid with free terminus

\[ F_n(s) = n(s) \cdot \int_s^L f^s(s')ds' = -n(s) \cdot \int_s^L \gamma \cdot \dot{R}(s')ds' \]  

(S6)

where \( \gamma = \gamma_n n + \gamma_t t \), \( \dot{R}(s) = \dot{R}_n(s)n + \dot{R}_t(s)t \). Thus in the tangent-normal frame of the filament,

\[ F_n(s) = -\gamma_n n(s) \cdot \int_s^L \left[ \dot{R}_n(s')n(s') + \frac{\dot{R}_t(s')}{2} t(s') \right] ds' = -\gamma_n g_n(s) \]  

(S7)

Thus,

\[ g_n(s) = n(s) \cdot \int_s^L \left[ \dot{R}_n(s')n(s') + \frac{\dot{R}_t(s')}{2} t(s') \right] ds' \]  

(S8)

In terms of the tangent angle \( \theta \), \( t(s) = [\cos(\theta(s)), \sin(\theta(s))] \) and \( n(s) = [-\sin(\theta(s)), \cos(\theta(s))] \). Hence \( \dot{R}_n(s) = -\sin(\theta(s))\dot{R}_x(s) + \cos(\theta(s))\dot{R}_y(s) \) and \( \dot{R}_t(s) = \cos(\theta(s))\dot{R}_x(s) + \sin(\theta(s))\dot{R}_y(s) \), where \( \dot{R}_x \) and \( \dot{R}_y \) are \( x \) and \( y \) components of filament velocity. As \( \partial_t \dot{R} = t, \dot{R} = \int_0^s [\cos(\theta(s')), \sin(\theta(s'))] ds' \) and

\[ \dot{R}(s) = \int_0^s -\sin(\theta(s'))\partial_t \theta(s')ds' \hat{i} + \int_0^s \cos(\theta(s'))\partial_t \theta(s')ds' \hat{j} \]  

(S9)

Ideally, the normal and tangential component of stress resultant are coupled to each other via the force balance equation \( F_n(s) = -\gamma_n \int_s^L \dot{R}_n(s')ds' + \int_s^L \kappa(s')F_t(s')ds' \) and one needs to solve the integral equation for \( F_t \) separately [22, 23, 63]. But in our case, we overcome that problem by being in the intrinsic arc-length parametrisation and knowing the filament velocity from its waveform measurement. Thus we can easily calculate the normal component of stress resultant without any small amplitude approximation. This is different from most of the previous works, where instead of solving the \( F_t \) equation, \( F_n \) was approximated by \( -\gamma_n \int_s^L \dot{R}_n(s')ds' \), which is the small curvature limit \[18, 21\]. We calculate every quantity in appropriate non-dimensional forms. Derivatives in time are calculated analytically whereas derivatives and integration in \( s \) are calculated numerically using the continuous Chebyshev interpolations of the amplitude and phase of the travelling wave in \( \theta \).

Tension forces in the filament: The tangential component of the stress resultant is the tension within the filament, which we can compute similarly from filament velocity as \( F_t(s) = -t(s) \cdot \int_s^L \gamma \cdot \dot{R}(s')ds' = -\gamma_n g_t(s) \), where, \( g_t(s) = t(s) \cdot \int_s^L \dot{R}_n(s')n(s') + (\dot{R}_t(s')/2) t(s')ds' \). The tension force in the filament is also too small (Fig. S2) compared to the internal elastic forces.

Boundary conditions of a clamped filament

For a filament which is clamped at the base, there is no sliding displacement at the base. Hence \( u(0) = 0 \implies \Delta \theta(0) = 0 \forall t \). At the free terminus of the filament, the net torque vanishes. Hence \( M(L) = EI\kappa(L) + \Gamma\kappa(L) = 0 \).

The curvature at the free end is thus,

\[ \kappa(L, t) = \kappa(L, 0) e^{-\frac{EI\kappa(L)t}{\nu_n}} = \kappa(L, 0) e^{-\nu_n t} \]  

(S10)

If we measure \( t \) in units of the beat frequency i.e., \( 1/\nu_0 \), then the curvature relaxation rate at the free end is \( \nu_0/\nu_n \approx 0.04 \). Hence \( \kappa(L) \to 0 \) within one quarter of the first beat cycle and we can approximate that \( \kappa(L, t) = 0 \forall t \). Thus the clamped head-free end boundary conditions of a filament are

\[ \begin{align*}
(i) & \quad \Delta \theta(0) = 0 \quad (\text{vanishing basal shear}) \\
(ii) & \quad \partial_{\theta} \Delta \theta(L) = 0 \quad (\text{vanishing terminal moment})
\end{align*} \]  

(S11)

Experimentally, the filament does not satisfy zero curvature at the distal end for the following reasons:

(a) The distal end of the filament goes out of focus most of the time, hence difficult to exactly track it.

(b) The \( s \)-derivatives are in Chebyshev modes, hence not reliable near the end points. As we express the tangent angle by a traveling wave and \( \kappa = \partial_{\theta} \Delta \theta \), we also get a traveling wave for \( \kappa \) with unreliable end values.
Linear Stability Analysis

The coupled equations of motion (Eq. 11 in main text) for the active filament is

$$\partial_t \left[ \left( \frac{\partial^2 - \nu_n/\nu_u}{m^4} \right) \Delta \theta \right] = \begin{bmatrix} 1 - \frac{\partial^2}{b_3} & -1 \\ b_3 & -b_1 \end{bmatrix} \begin{bmatrix} \Delta \theta \\ m^4 \end{bmatrix}$$

(S12)

We Fourier transform the above equations in space and time with the following convention

$$\Delta \theta(s, t) = \int \frac{dz}{2\pi} \sum q \Delta \bar{\theta}(q_n, s) e^{i(q_n s - z t)}$$

where $q_n$ is the discretized wavenumber of the $n$th mode due to finite length of the filament and $z$ is the complex frequency. In matrix form,

$$\begin{bmatrix} (1 + q_n^2) - iz(q_n^2 + \nu_n/\nu_u) & -1 \\ b_3 & iz - b_1 \end{bmatrix} \begin{bmatrix} \Delta \bar{\theta} \\ m^4 \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \end{bmatrix}$$

(S13)

For non-trivial solution, the determinant of the above matrix must be zero. Hence the dispersion relation is

$$\left[ (1 + q_n^2) - iz(q_n^2 + \nu_n/\nu_u) \right] (iz - b_1) + b_3 = 0$$

(S14)

This is a quadratic equation in the complex frequency $z$ of the form $A(q_n)z^2 + B(q_n)z + C(q_n) = 0$ whose coefficients are:

$$A(q_n) = q_n^2 + \nu_n/\nu_u, \quad B(q_n) = i \left[ (1 + q_n^2) + b_1(q_n^2 + \nu_n/\nu_u) \right], \quad C(q_n) = b_3 - b_1(1 + q_n^2)$$

(Roots are $z_{1,2} = [-B(q_n) \pm \sqrt{B^2(q_n) - 4A(q_n)C(q_n)}]/2A(q_n)$. As the time dependent component in the solution is $e^{-izt}$, existence of the real part of the root will imply that the solution is oscillatory and sign of the imaginary part of $z$ will decide if the solution is growing or decaying. Hence, the conditions for unstable oscillations are $Im|z| > 0$ and $Re|z| \neq 0$. The frequency of the oscillations is therefore given by $\omega = Re|z|$.

Allowed wavenumbers by the filament boundary condition: If we consider only the Fourier transform of the coupled equation in time, it is a harmonic oscillator equation in $s$-space. The solutions are (sin, cos). Using the clamped head-free end boundary conditions of the filament (Eq. S11), the shear angle has a solution of the form $\Delta \theta(s) \sim \sum_{n=odd} \sin(q_n s)$, where the allowed modes are $q_n = \frac{n \pi}{2 \ell / l_n}$, $n = 1, 3, 5...$ (odd).

Elastic and viscous components of active stress

We have shown that the coupled equation of motion has unstable oscillatory solutions for $b_1, b_3 < 0$. Hence, active moment coupled to the shear angle as $\partial m^4 + b_1 m^4 = b_3 \Delta \theta$ is also oscillatory in this regime. Therefore, if we consider the fundamental Fourier mode as $m^4 = m^4 e^{i\omega t}$ and $\Delta \theta = \Delta \bar{\theta} e^{i\omega t}$ (similar to [21], where $\omega$ is real), then the active stress has a form of Kelvin-Voigt model of viscoelasticity.

$$\bar{m}^4 = \frac{b_3}{b_1 + i\omega} \Delta \bar{\theta} = (G' + i\omega G'') \Delta \bar{\theta}, \quad G' = \frac{b_1 b_3}{b_1^2 + \omega^2}, \quad G'' = \frac{-b_3}{b_1^2 + \omega^2}$$

(S16)

The elastic ($G'$) and viscous ($G''$) response of the active stress are governed by the control parameters, $b_1$ and $b_3$. The sign of response coefficients determine the nature of active stress*.

*The sign convention of $G'$ and $G''$ is opposite to the elastoviscous response coefficients $(K$ and $\lambda$) of [21, 30, 51], as active drive in these references has opposite sign, described in the next section.

Near the instability threshold: The system, operating near the instability threshold, spontaneously oscillates at frequency of $\omega_{expt} = 2\pi \nu_b/\nu_u \approx 0.272$. This limit cycle frequency from the linear analysis (near the threshold $\omega = Re|z|$) fixes the active model parameters $b_1 = -0.121, b_3 = -0.85$ for the first oscillatory mode ($q_1$). Thus the active elastic and viscous response coefficients are $G' \sim 1.16$ and $G'' \sim 9.6$ respectively. As $G'' > G'$, the viscous response dominates over the elastic one in our experiment.
Comparison with microscopic load dependent detachment model of dynein motors

Riedel-Kruse et al. have proposed Kelvin-Voigt model of active stress (also called sliding control motor coordination) for sperm flagellum [21], where the active shear force per unit length \( f \) is related to the shear displacement \( \Delta \) by a response function, \( \chi \), as per their notation. The equivalent notation of our work with [21], considering only the active contribution of shear force is \( m^A = -af \), \( a\Delta \theta = \Delta \). The relation between the fundamental Fourier modes of the active drive and shear displacement of [21] in our notation is as follows:

\[
\text{Eq. (5) of [21]: } \tilde{f}(s) = \chi \tilde{\Delta}(s) = (K + i\omega \lambda)\tilde{\Delta}(s) \quad (S17)
\]

In our notation:

\[
\tilde{m}^A(s) = -a^2\chi \tilde{\Delta}(s) = -a^2(K + i\omega \lambda)\tilde{\Delta}(s) \quad (S18)
\]

Replacing \( K \) and \( \lambda \) from the microscopic model of load dependent detachment model of motors (Eq. B9 in Appendix B of [21])

\[
\tilde{m}^A(s) = -a^2\left(-2\rho \tilde{f}\left(1 - \bar{p}\right)\frac{\omega^2\bar{\tau}}{1 + (\omega\bar{\tau})^2} + i\omega 2\rho f'\bar{p}\left[1 - \frac{\tilde{f}}{f_c}\frac{(1 - \bar{p})}{1 + (\omega\bar{\tau})^2}\right]\right)\tilde{\theta}(s) \quad (S19)
\]

where, \( \rho \) is the motor density, \( \tilde{f} \) is the dynein stall force, \( f' \) is the slope of the motor force-velocity curve, \( f_c \) is a characteristic force (an increase in the load/dynein force by \( f_c \) increases the detachment rate by \( e \) fold), \( \bar{p} \) is the fraction of motor domains that are bound to the microtubule, \( \bar{\tau} \) is the relaxation time of motor attachment/detachment. The equivalence of active elastoviscous response coefficients of ours with [21] is as follows:

| Model in this work | Microscopic model in [21] |
|--------------------|---------------------------|
| \( G' = \frac{b_1 b_3}{b_1^2 + \omega^2} \) | \(-a^2K = 2a^2\rho f'\left(1 - \bar{p}\right)\frac{\omega^2\bar{\tau}}{1 + (\omega\bar{\tau})^2} \) |
| \( G'' = \frac{-b_3}{b_1^2 + \omega^2} \) | \(-a^2\lambda = -2a^2\rho f'\bar{p} \left[1 - \frac{\tilde{f}}{f_c}\frac{(1 - \bar{p})}{1 + (\omega\bar{\tau})^2}\right] \) |

*As shown in the table \( G', G'' \) are related to \( K, \lambda \) by a minus sign. [21] finds oscillatory solutions when \( K, \lambda < 0 \). We find oscillations when \( G', G'' > 0 \) implying that the results are consistent with [21].

All of the quantities in the active elastic response of the equivalent microscopic model, \( G' = 2a^2\rho f'\left(1 - \bar{p}\right)\frac{\omega^2\bar{\tau}}{1 + (\omega\bar{\tau})^2} \), are positive. Hence \( G' > 0 \) tallies with the microscopic model of load dependent detachment rate of motors. The sign of the viscous response coefficient, \( G'' \equiv -a^2\lambda = -2a^2\rho f'\bar{p} \left[1 - \frac{\tilde{f}}{f_c}\frac{(1 - \bar{p})}{1 + (\omega\bar{\tau})^2}\right] \), depends on the relaxation time and fraction of motor domains bound to the microtubule. Specifically,

(a) \( G'' > 0 \) for \( \omega\bar{\tau} \ll 1 \) and \( \bar{p} \sim 0 \),
(b) \( G'' < 0 \) for \( \omega\bar{\tau} \gg 1 \) and \( \bar{p} \sim 1 \).

The fact that \( G'' > 0 \) i.e. \( b_3 < 0 \) asserts that for our active filament, \( \omega\bar{\tau} \ll 1 \) and \( \bar{p} \sim 0 \). This means that the axonemal dynein motors are short lived with low duty ratio.
### MECHANICAL PARAMETERS OF THE AXONEME

| Parameter | Value | Units | Description and Reference |
|-----------|-------|-------|---------------------------|
| $L$       | 10.935 | µm    | length of axoneme (Fig. 1a) |
| $a$       | 0.2   | µm    | diameter of axoneme [28]    |
| $\nu_b$   | 16.22 | Hz    | beat frequency* (movie S1)  |
| $EI$      | $0.6 \times 10^{-21}$ Nm² | bending rigidity of flagellum [23, 64, 65] |
| $k$       | $2 \times 10^3$ N/m² | linear shear stiffness of axoneme [44, 65] |
| $\gamma_n$ | 4.35 | mPa.s | normal hydrodynamic drag coefficient** [66, 67] |
| $\Gamma_k$ | $1.6 \times 10^{-24}$ Nm²s | bending friction coefficient [42, 43] |
| $\Gamma_u$ | $10 \times 10^{-6}$ Ns/m | shear friction coefficient*** [46] |

* Average over three beat cycles, for which the space-time graphs are plotted

**For a slender cylinder ($L >> a$) near a planar boundary, $\gamma_n = \frac{4\pi\eta}{ln(4h/a)}$, $\eta = 1$ mPa.s being the fluid viscosity and $h = 0.9$ µm being the height of the filament centreline above the planar surface.

*** Not experimentally measured, estimation from nexin protein friction.

TABLE I. Mechanical parameters of the axoneme with their references.

| Parameter | Value | Description | Comparison |
|-----------|-------|-------------|------------|
| $l_\kappa = \sqrt{EI/a^2k}$ | 2.74 µm | curvature penetration length scale | $L/l_\kappa = 4$ |
| $\nu_h = EI/\gamma_n l_\kappa^4$ | 2447 Hz | hydrodynamic relaxation frequency scale | $\nu_h/\nu_b = 151$ |
| $\nu_n = EI/\Gamma_k$ | 375 Hz | curvature relaxation frequency scale | $\nu_n/\nu_b = 23.12$ |
| $\nu_u = ak/\Gamma_u$ | 40 Hz | shear relaxation frequency scale | $\nu_u/\nu_n = 9.38$ |

TABLE II. Length and frequency scales of the system

### Estimates of $\Gamma_u$ from literature

All parameters, except the shear friction coefficient, have been experimentally measured for an axoneme or at least for microtubules in the existing literature as mentioned in Table I. There is some uncertainty in $\Gamma_u$. Following are the values used for this coefficient for constructing active filament models in the literature, except for the last entry which is an experimental study.

| Reference            | $\Gamma_u \times 10^{-6}$ Ns/m | Comparison of shear friction with elasticity |
|----------------------|---------------------------------|---------------------------------------------|
| Brokaw [47]          | 0.06-0.51                       | negligible compared to elastic terms        |
| Murase-Shimizu [49]  | 30                              | very high compared to elastic terms, overdamping the system |
| Bayly-Wilson [68]    | 0.5                             | negligible compared to elastic terms        |
| Bayly-Dutch [50]     | 0.16                            | negligible compared to elastic terms        |
| Ingmar Riedel [46]   | 10                              | comparable and competing with elastic terms, ✓ |
| Minoura-Yagi-Kamiya [44] | 365                        | very high compared to elastic terms, overdamping the system* |

* obtained from digitizing Fig. 5, and fitting a creep response function for Kelvin Voigt model of viscoelasticity.

TABLE III. Shear friction coefficient from existing literature and reasons for neglecting/accepting them.
## COMPARISON OF DIFFERENT ACTIVE FILAMENT MODELS IN THE EXISTING LITERATURE

| Reference                  | External viscous drag | Moment resultant | Passive moment/length | Active drive |
|---------------------------|-----------------------|------------------|-----------------------|--------------|
| Machin [69, 70]           | $dF_e = \beta \partial_y y dx$ | $dF_e = -\alpha \partial^2_y y dx$ | $m = r \alpha_2 u$ | $R = \Phi \tilde{\partial}^2_y y$ |
| Brokaw [18]               | $d^2 M_e = -C_N V_N$ | $M = -2 \alpha_0 k$ | $m = (1 + 2 \alpha_0 / \alpha_1) S$ | $m = h^2 \alpha_2 \gamma_s$ |
| Lubliner-Blum [71]        | $W \equiv \partial^2_t M_e = \kappa \eta V$ | $M = 2 \alpha_0 \mu$ | $m = (1 + 2 \alpha_0 / \alpha_1) S$ | $m = h^2 \alpha_2 \gamma_s$ |
| Brokaw* [47]              | $dF_N = -C_N V_N ds$ | $M = -E_B \kappa - C_B \kappa$ | $m = E_S \sigma + C_S \sigma$ | $m(t) = -m_0 \kappa (t - \tau)$ |
| Hines-Blum [48]           | $\phi_N = -C_N V_N$ | $M = -E_b \kappa$ | $S_r \sim \lambda_r \gamma$ | $\tau \partial S_d = -m_0 \kappa - S_d$ |
| Rikmenspoel [19]          | $dM_i = -k(\xi - x) \delta U(\xi)$ | $M_{el} = E1 \partial^2 U$ | $m = l^2 \tilde{m}_0 (t - x - \delta)$ | $M_a = e^{l^2 \tilde{m}_0 (t - x - \delta)}$ |
| Hines-Blum [20]           | $\phi_N = -C_N V_N$ | $M = -E_b \delta \alpha$ | $S = -E_r \gamma$ | $\tau \partial S_d = -m_0 \kappa - S_d$ |
| Murase-Shimizu** [49]     | $f^v = 0$ | $M = E1 \delta \alpha$ | $m = P_H - h \gamma \delta \alpha$ | $m = F_A + F_B$ (Eq. 2) |
| Camalet-Julicher [30]     | $f^v = -\xi \cdot \partial_t r$ | $M = \kappa C$ | $m = -af$ | $\tilde{f} = \chi(\Omega, \omega) \Delta$ |
| Riedel-Kruse et al. [21]  | $f^v = -\xi \cdot \partial_t r_n$ | $M = \kappa \delta \alpha$ | $m = -af$ | $\tilde{f}(s) = \chi(\Omega, \omega) \Delta(s)$ |
| Gaffney et al. [10]       | $\lambda \partial_t f_N + f_N = -C_N U_N$ | $M = E(s) \kappa(s)$ | $m(s) = b(s) f(s)$ | $	ilde{f} = \chi(\Omega, \omega) \Delta$ |
| Bayly-Wilson [68]         | $q_N = -C_N V_N$ | $M_B = E1 \partial \psi$ | $f_T = -a(k_T \psi + b_T \psi + a^2_T \psi^3)$ | $f_T = f_T p$ (Eq. 29) |
| Bayly-Wilson [22]         | $q_N = -C_N V_N$ | $M_B = E1 \partial \psi$ | $m = -a(f + k_1 \Delta^2)$ | (Eq. 3.6, 3.20, 3.25) |
| Bayly-Dutcher [50]        | $q_N = -C_N V_N$ | $M_B = E1 \partial \psi$ | $m = -a^2(k_T \psi + b_T \psi + a^2_T \psi^3)$ | $f_T = p D(s)$ |
| Sartori et al. [23]       | $f^v = -\xi \cdot \partial_t r$ | $M = \kappa \delta \alpha$ | $m = -af$ | $f_n(s) = \beta(\Omega \delta \alpha) \psi_n(s)$ |
| Ishimoto-Gaffney [24]     | $f^{hyd}$ (Eq. 2.13, 2.14) | $M^v = a_1 K_i$ | $F^v = b_1(D^\eta \partial \psi - \delta)\Delta$ | $m^{int} \sim \sin(ks - \omega t) D^\eta$ |
| **Our model**             | $f^v = -\gamma \cdot R$, non-existent | $M = E1 \Gamma + \Gamma_x \kappa$ | $m = -a \kappa - \Gamma_x \tilde{u}$ | $\partial_t m^4 + b_1 m^4 = b_3 \Delta \theta$ |

*Internal viscous resistances, necessary, only to stabilize numerical simulation, proved by [48].

**Dropped external viscosity from theoretical estimations and waveform measurements on sperm flagella (not validated the drag from flow measurements); included internal shear viscosity only to reduce numerical complexity.

#### TABLE IV. Comparison of active filament models in the framework of the exact theory of stress and strain in rods.

## MOVIE CAPTION

**Movie S1:** High speed phase contrast video of a reactivated and clamped *Chlamydomonas* axoneme at 60 μM ATP in presence of 200 nm tracer particles. Scale bar, 5 μm.
FIG. S1. **Chebyshev differentiation for different orders.** (a) Amplitude ($\theta_0$) and phase ($\phi$) of the travelling wave parametrization to $\theta$ are plotted in cyan circles. Interpolation to them for different Chebyshev polynomial orders, almost converges to the actual value. (b) First order Chebyshev differentiation of $\theta_0$ and $\phi$ with respect to $s$ for different polynomial orders $N$. (c) Second order Chebyshev differentiation of $\theta_0$ and $\phi$ with respect to $s$ for different polynomial orders $N$. It is evident that the Chebyshev derivatives oscillates near the end (more so for the 2nd order derivatives), hence unreliable at those points. In between the dashed and dotted lines in (b) and (c) the values of derivatives for different orders converge. Hence, we consider $s/L \in [0.1, 0.9]$ when considering derivatives higher than 1st order, and neglect the end values.
FIG. S2. **Tension forces in the filament.** Space-time plot of the tension force i.e. tangential component of the stress resultant, along the filament for three beat cycles. The length and time scales are $l_\kappa$ and $1/\nu_h$. The colorbar represents its magnitude, with the force scale $EI/l_\kappa^2 = 80$ pN.
FIG. S3. **Active filaments driven by slip at boundary vs driven internally by motors.** (a,b) Flow fields of a planar flexible beating of a clamped filament, consisting of chemomechanically active beads, at two instants of the oscillation cycle, adapted from Supplementary video 2 in [54]. (c,d) Computed flow fields using slender body approximation and resistive force theory (unbounded flow) where the filament positions were extracted from the video. The mismatch between (a)-(c) and (b)-(d) imply that the filament must not be internally driven instead slip driven as expected of a phoretic chain. The colorbars to the right of (c), (d) represents the normalized speed.