Probing microtubule-kinesin active matter in a low activity regime

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(Dated: October 1, 2022)

Countless living systems exhibit complex behaviors driven by the spontaneous self-organization of their constituents. The dynamics of these systems occur far from equilibrium and often rely on the localized consumption of biological energy sources. In this work, we leverage kinesin-driven microtubule networks as a simple experimental system to quantify non-equilibrium material behaviors. By limiting kinesin-motors' access to energy-rich ATP, we tune the activity of our reconstituted ensembles, allowing us to probe the transition between fluidized and elastically gelled protein suspensions. We observe that our system's dynamics depend non-trivially on ATP concentration and can be further tuned by varying an incorporated ATP regeneration backbone. Together, these observations lend a new perspective on the energy-consuming protein interactions that drive emergent non-equilibrium flow in active materials.

I. INTRODUCTION

Many-bodied soft matter systems, driven by thermal fluctuations, often behave as active materials. Energy consumption in active matter occurs on the level of constituent parts, whose motion drive emergent non-equilibrium dynamics and intricate collective behaviors. Inherently out-of-equilibrium biological systems are abundant in nature, including bird flock dynamics, bacterial swarming, and cellular cytoplasmic streaming phenomena. However, these systems are difficult to manipulate and therefore study directly.

Microtubule-kinesin active matter, comprised of materials found in cell cytoplasmic environments, serves as a model active system. The tunable dynamics of these active materials provides an ideal system for studying emergent non-equilibrium dynamics. In particular, the activity of microtubule-kinesin active materials is tied to kinesin motor adenosine triphosphate (ATP) consumption. The speed of the material is tuned by ATP concentration, increasing initially until reaching a plateau after the ATP concentration saturates. Tuning ATP concentration therefore changes the activity of the active material [1].

This project sought to probe microtubule-kinesin active materials in a low activity regime where kinesin motors cannot access ATP as readily as in systems at saturated ATP concentrations. We vary both ATP concentration and ATP regeneration rate and observe the emergent material behavior. Our results show a transition in the behavior of microtubule-kinesin active materials as activity decreases. This transition is evident both in the motion and structure of the material.

A. Microtubule-kinesin active materials

Microtubule-kinesin active materials are composed of cytoskeletal proteins. The key components are microtubules, polar filaments polymerized from dimerized α- and β-tubulin; kinesin motor clusters, artificially formed constructs of biotinylated kinesin motors and streptavidin protein (KSA); and ATP, cellular energy-carrying molecules. Individually, kinesin motors consume energy through ATP hydrolysis, releasing chemical energy used to walk along microtubules. Kinesin-1 motors specifically walk toward the plus ends of microtubules. The formation of KSA allows coupling between motors walking along separate microtubules. In the instances when kinesin-1 motor clusters walk along anti-parallel microtubules, the microtubules slide past each other, exhibiting extensile behavior. However, if kinesin motors cannot readily access ATP, the motors remain bound to microtubules and act as cross-linkers between them. Collectively, the combination of countless of these subunits generates a fluidized active material, and energy consumption by kinesin motors drives emergent non-equilibrium dynamics.

The phosphate bonds between adenosine diphosphate (ADP) and lone phosphate groups do not reform spontaneously. The regeneration of ATP hydrolyzed by kinesin motor activity is catalyzed by the enzyme pyruvate kinase [2]. Pyruvate kinase uses phosphoenol pyruvic acid (PEP) as an energy source to catalyze the reformation of ATP, ensuring the concentration of ATP is constant over time. The active dynamics of the sample are maintained for hours so long as the reserve of PEP is not exhausted [3]. The kinesin motor-driven activity of the material is therefore consistent over time.

II. METHODS

The active gel preparation is suspended in an M2B buffer solution. Microtubules are polymerized in solution, and the addition of poly(ethylene glycol), or PEG, depletes polymerized microtubules into nematically-aligned bundles. A portion of the tubulin polymerized is labeled with Alexa 647 dye for observation. K401 is a truncated version of kinesin-1 containing only the first 401 amino acids of kinesin-1. Biotinylated K401 motors are bound to the protein streptavidin and form KSA dimers by leveraging biotin's strong affinity for streptavidin. Kinesin motors in solution bind to and hydrolyze ATP as a fuel source to walk along microtubule bundles. The hydrolysis product ADP is resynthesized into ATP by a pyruvate kinase-based regeneration system. Pyruvate kinase uses PEP as an energy source to catalyze ATP resynthesis, maintaining a consistent ATP concentration over extended time periods. Fluorescent 3μm polystyrene tracer beads are coated with Alexa 488 dye and added in solution for particle tracking. The addition of antioxidants prevents photobleaching while imaging.

The active gel is flowed into 100μm Parafilm chambers between a polyacrylamide coated glass slide and coverslip. Due to the relatively small aspect ratio of the chamber height, the microtubules are shear-aligned along the pipetted flow. The active gel is imaged in the chambers using epifluorescence microscopy.

We varied the activity of the material by tuning the ATP available for consumption by kinesin motors. We first tuned activity by systematically decreasing the concentration of ATP added to the active gel. We then alternately tuned activity by decreasing pyruvate kinase concentration, therefore decreasing the rate of ATP regeneration.

III. RESULTS

We quantified the material dynamics by imaging fluorescent polystyrene beads suspended in the active gel and advected by the flow. We characterize the flow dynamics of the active material by measuring the mean-squared displacement (MSD) of bead trajectories. First, we determined how the dynamics of emergent flows changes with decreasing ATP concentrations. The measured mean-squared displacement (MSD) was dependent on changing ATP concentration (figure 1).

The slope of the MSD curves monotonically decreased as ATP concentration decreased. Gels with higher ATP concentrations moved ballistically over all measured timescales. Gels with lower ATP concentration moved diffusively over short timescales and ballistically over long timescales. The transition from diffusive motion to ballistic motion occurs over longer timescales as ATP concentration decreases.

Active gels at low ATP concentrations form bent sheets of microtubules. Confocal images of active gels with low

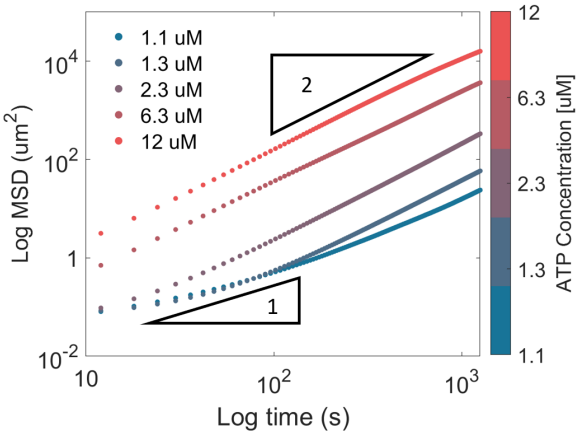


FIG. 1: Log mean-squared displacement vs. log timescale for decreasing ATP concentrations. The motion of the material shifts as ATP concentration and therefore activity decreases. Reference slopes of 1 for diffusive motion and 2 for ballistic motion are shown.

ATP concentrations show disparate regions of high and low microtubule density (figure 2). Fluorescent beads are more common is regions of high microtubule density than in vacuous regions of low microtubule density. The distribution of beads implies that the fluorescent beads are embedded in regions of high microtubule density. In contrast, beads in high ATP flows are free to move about the gel. The constraint of beads in specific regions at low activity suggests an increase in the viscoelastic nature of the material.

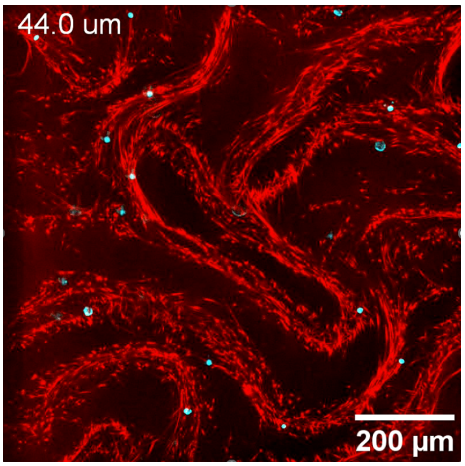


FIG. 2: Confocal cross-section of an active gel at 1μM ATP. Bundles of microtubules are red and fluorescent tracer beads are cyan. At low activity, the material forms sheets with dense regions of microtubules. Fluorescent beads are embedded in regions of high microtubule density.

When not actively bound to nucleotide, kinesin motors aggregate, an irreversible process which prevents

the stepping function of kinesin motors. This biochemical constraint prevents the construction of an active gel without the prescence of ATP. KSA stocks are therefore stored with ATP that cannot be fully removed without kinesin motor aggregation, limiting the minimum ATP concentration in an active gel preparation. To explore the material at low activity, we instead tuned the concentration of pyruvate kinase, therefore tuning the rate of ATP regeneration and effectively controlling ATP concentration without impacting nucleotide concentration.

We determined how the dynamics of emergent flows changes with decreasing pyruvate kinase concentrations. The measured MSD was dependent on changing pyruvate kinase concentration (figure 3). The slope of the MSD curves monotonically decreased as pyruvate kinase concentration decreased. Gels with higher pyruvate kinase concentrations moved ballistically over all measured timescales. Gels with lower pyruvate kinase concentration moved diffusively over short timescales and ballistically over long timescales. The transition from diffusive motion to ballistic motion occurs over longer timescales as pyruvate kinase concentration decreases.

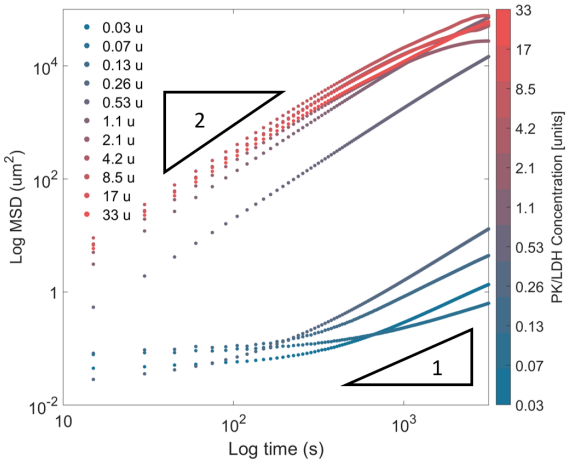
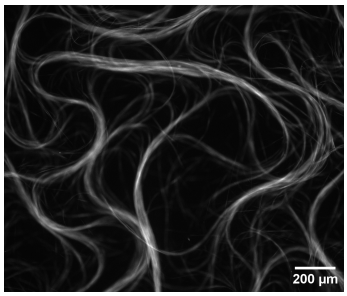
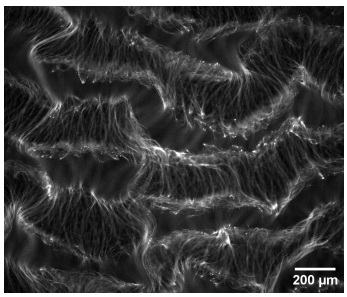


FIG. 3: Log mean-squared displacement vs. log timescale for decreasing pyruvate kinase concentrations. The motion of the material shifts as pyruvate kinase concentration and therefore activity decreases. Reference slopes of 1 for diffusive motion and 2 for ballistic motion are shown. One unit of pyruvate kinase corresponds to 1mM ATP regenerated per minute.

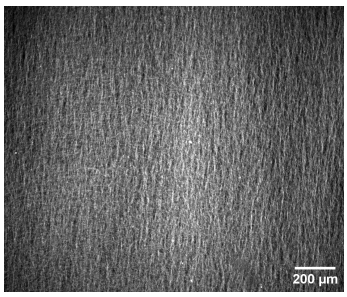
Active gels with decreasing pyruvate kinase concentrations show a distinct transition in the material behavior. At high concentrations of pyruvate kinase, a fluidized flow emerges (figure 4a). Within this flow, steady-state dynamics typical of saturated ATP gels emerge. As pyruvate kinase concentration decreases, an elastically gelled material emerges (figure 4b). The gel forms a dense sheet of microtubules with distinct bend patterns. At even lower pyruvate kinase concentrations, the microtubules remain shear-aligned (figure 4c), implying that there was no activity in the material.



(a) Fluidized active dynamics



(b) Elastically gelled material

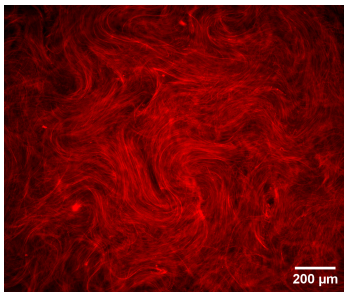


(c) Shear-aligned microtubules

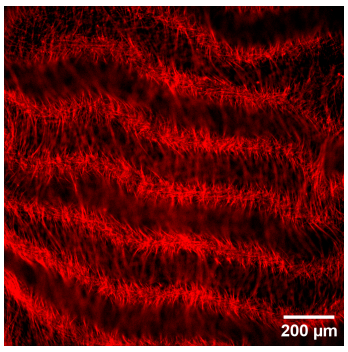
FIG. 4: The behavior of active gels shifts as pyruvate kinase concentration, and therefore activity, decreases. (a) At high pyruvate kinase concentrations, the microtubules reach steady-state fludized dynamics. (b) At low concentrations, elastically gelled microtubule sheets with distinct bends form. (c) At very low concentrations, the microtubules remain shear-aligned.

The emergent structure of microtubule-kinesin active matter takes on increasingly elastic properties as the concentration of available ATP decreases. As activity decreases, the active gel transitions from a fluidized material typical of saturated ATP flows to an elastically gelled material. Both at low ATP and low pyruvate kinase concentrations, a viscoelastic gel emerges with the formation of a dense sheet of microtubules with distinct bend instabilities.

The transition in material behavior at low ATP and pyruvate kinase concentrations corresponds to kinesin motor stepping activity. The stepping activity of individual motors is related to the availability of the energy source ATP. Kinesin motors hydrolyze ATP to walk along microtubules. Between steps, kinesin motors re-



(a) Fluidized material



(b) Elastically gelled material

FIG. 5: Transition from fluidized material to elastically gelled material as activity decreases. (a) At high activity, a fluidized material emerges. (b) At low activity, an elastically gelled material emerges.

main bound to ADP nucleotides before hydrolyzing a new ATP molecule [4]. During this transition state, kinesin motor clusters remain bound to the microtubules and act as cross-linkers holding microtubule bundles together. As ATP or pyruvate kinase concentration decreases, the availability of ATP to kinesin motors decreases and therefore kinesin motors step with decreasing frequency. We propose that the viscoelastic character of low activity gels corresponds to the interplay of kinesin motor stepping and cross-linking behaviors as ATP availability decreases.

IV. DISCUSSION

Microtubule-kinesin active gels are hierarchically structured materials that exhibit inherently out-of-equilibrium emergent dynamics [2]. The tunable nature of such active materials has been studied in depth, as well as the activity of kinesin motors [1, 5]. However, a detailed exploration of microtubule-kinesin active matter in a low activity regime has not yet been performed.

Dictated by kinesin motor cluster energy consumption, the characteristics of microtubule materials is highly tunable by the concentration of available energy-rich ATP molecules. We find that the fluidized active flows at high activity transition to an elastic material at low activity. Kinesin motors in this regime consume discrete energy molecules with decreasing frequency, resulting in a decrease in stepping frequency. The cross-linking state of kinesin motors between steps ultimately contributes to elastic material properties at low activity.

ACKNOWLEDGMENTS

I would like to thank Dr. Zvonimir Dogic for advising this project, my graduate mentor Rémi Boros for his enormous support both during and after the REU program, and REU Site Director Dr. Sathya Guruswamy for organizing the REU program and inviting me to be a part of the 2022 cohort. I also thank Isabel Ruffin for training me on fluorescence and confocal microscopy, Chris Ramirez for helping me prepare acrylamide slides, and Dr. John Berezney, Sattvic Ray, and Nicholas Cuccia for their guidance during the writing process along with the remaining members of the Dogic Group for their support. This project would not have been possible without the generous support of everyone involved. This work was supported by the National Science Foundation, Division of Physics under award NSF-PHY-1852574.

Appendix A: Application of MSD

It is unfeasible to individually track and measure the countless constituents contained in a single sample of microtubule-kinesin active matter. Particle tracking on fluorescent polystyrene tracer beads advected by the active sample is instead performed to measure the collective dynamics. The material generally moves with random, diffusive motion or directed, ballistic motion in reference to a given timescale. Particles moving diffusively collide with surrounding molecules randomly with no preference for direction. Particles moving ballistically are acted on by a directed force, generating particle motion in a correlated direction with its neighbors. Calculating the mean-squared displacement (MSD) of particle trajectories aids in characterizing the material dynamics.

MSD is a statistical mechanical measure of a particle's deviation in position compared to a reference position over time and therefore the area a particle explores. MSD is calculated as the average of the squared displacements of particles over time intervals of varying size, rather than as a function of time duration. Equation A1 shows the calculation of MSD for one timescale.

$$MSD = \frac{1}{N}\sum_{i=1}^{N} |x^{(i)}(\Delta t) - x^{(i)}(t_0)|^2 \qquad \text{(A1)}$$

The position of the particle after some time interval Δt, $x^{(i)}(\Delta t)$, is compared to the reference position of the particle, $x^{(i)}(t_0)$. The time interval given by Δt denotes the timescale of the measurement. The number of squared displacements averaged is denoted by N.

The MSD of diffusive particles depends linearly on the timescale while the MSD of ballistic particles depends on the square of the timescale. The power law nature of diffusive and ballistic motion is extracted by comparing the logarithm of MSD to the logarithm of the timescales. In a log-log relationship of MSD vs. timescale, diffusive motion emerges as a linear trend with slope 1 and ballistic motion emerges as a linear trend with slope 2. Figure 6 shows this relationship for model diffusive and ballistic motion. Analysis of the rate of change of log MSD vs. log timescale therefore provides a quantitative means of understanding the material dynamics.

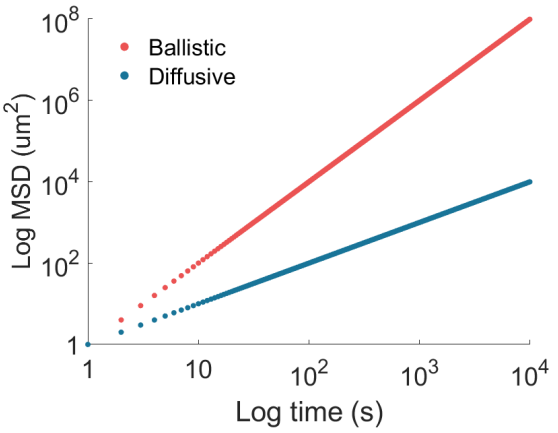


FIG. 6: Model log-log plot of mean-squared displacement vs. time. Diffusive motion follows a linear trend with a slope of 1 while ballistic motion has a slope of 2.

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