

3D Agent-Based Modeling without Chemical Signaling Recreates Collective Behaviors seen in *Myxococcus xanthus* Colonies

Katherine Copenhagen ¹, Matthew Black,¹ and Joshua W. Shaevitz ^{1,2,*}

¹*Lewis-Sigler Institute of Integrative Genomics, Princeton University, Princeton, New Jersey, USA*

²*Joseph Henry Laboratory of Physics, Princeton University, Princeton, New Jersey, USA*



(Received 23 May 2025; accepted 30 September 2025; published 1 December 2025)

Myxococcus xanthus is a soil-dwelling bacterium that lives in dense populations of millions of cells and displays an array of collective behaviors and macroscopic patterns in response to environmental conditions. The transitions between macroscopic patterns formed by this species are driven by changes in cell motility such as increasing Péclet number via increasing cell speed and reversal period. While cells are capable of passing contact-mediated signals that can trigger reversal events, we set out to study which aspects of *M. xanthus* collective behavior may be driven by simple individual cell behavior changes in the absence of direct chemical cell signaling. We do this through three-dimensional agent-based simulations with a bead-spring chain model. We find that when tuned to match single-cell properties, this model captures multicellular structures observed at both intermediate and global scales in *M. xanthus* experiments. Increasing cell speed and reversal period together (as cells do when they starve) maintains a constant overall nematic structure in the population, while significantly increasing the net flow into and out of topological defects, which drives an increased aggregation that leads to fruiting-body formation during starvation.

DOI: [10.1103/jfqj-2n4q](https://doi.org/10.1103/jfqj-2n4q)

I. INTRODUCTION

Dense populations of reversing self-propelled rods are a class of active materials, prevalent in nature from bacterial colonies to sea algae. These collectives display a wide variety of unique behaviors and patterns due to their combination of polar and nematic features [1]. Microscopic interactions between self-propelled rods are typically nematic, i.e., head-tail symmetric, driven by steric collisions and volume exclusion as rods tend to align their long axes when packed together. Their direction of self-propulsion, however, endows particles with a natural polarity. Because of this natural polarity, groups of self-propelled rods moving in the same direction create local polar order through polar sorting [2–4]. This accumulation of local polar order has been shown to create both traveling waves in groups of swimming bacteria [3] and, in *Myxococcus xanthus*, accumulation of local stresses leading to the out-of-plane extrusion of cells [5].

Local polar order, however, is interrupted when some fraction of the cells in a group reverse their direction of motion. At the single-cell level, directional reversals offer a means of modulating their effective diffusivity [6,7]. Tuning the period at which such reversals take place provides an internally controllable timescale by which cells can “switch” between ballistic and diffusive motion [8]. Such directional reversals

appear to be exploited throughout the biological world. Neural progenitor cells have been shown to exploit reversals to promote cell aggregation [9]. Several marine species of bacteria and algae self-propel by flagellar-mediated swimming while undergoing periodic directional reversals [10,11]. Terrestrial bacteria, which move by either pilus-based twitching or focal-adhesion-based gliding, also may exhibit directional reversals [12–15].

The soil bacterium *M. xanthus* is an ideal model organism for studying the relationship between single-cell properties and population-level collective behaviors and pattern formation in systems of self-propelled reversing rods. Single *M. xanthus* cells are about 0.5 μm wide and 5 μm long and glide across surfaces at a speed of about a body length per minute, reversing their direction of motion approximately every 8 minutes. On the other end of the scale, in populations of millions of cells, *M. xanthus* exhibits a complex multicellular life cycle, forming different collective three-dimensional (3D) structures depending on environmental conditions [16]. For example, groups of *M. xanthus* cells predate on other bacteria and exhibit “rippling” waves, nutrient-starved populations aggregate into macroscopic, spore-filled fruiting bodies, while well-fed populations spread out into a thin swarming layer of cells coating a surface [17]. Transitions between these different population structures are driven by changes in motility at the single-cell level [18], which may be triggered by local cell-cell contacts [19] or individual cells sensing local nutrient conditions and internal state [20]. Understanding the full physical mechanisms connecting single-cell behavior to population-level structure formation will increase our knowledge of the *M. xanthus* life cycle and also reveal simple single-particle properties that may be exploited to drive active materials between different macroscopic phases.

*Contact author: shaevitz@princeton.edu

Published by the American Physical Society under the terms of the [Creative Commons Attribution 4.0 International](https://creativecommons.org/licenses/by/4.0/) license. Further distribution of this work must maintain attribution to the author(s) and the published article's title, journal citation, and DOI.

Individual cells respond to nutrient stress by both moving faster and increasing the time between reversal events (reversal period). At the population level, these motility changes cause an apparent spinodal decomposition from a thinly spread layer to a collection of well-separated fruiting bodies [18]. Recent work shows that, on an intermediate length scale, well-fed cells form a thin swarm and pack together, aligning their long axes over a range of several cell lengths. As cells glide smoothly past each other within the cell layers, they exert pushing forces along the cell alignment direction. The population thus forms an active nematic material, and internal patterns form where cells become misaligned at sites called topological defects [21,22].

During the initial stages of fruiting-body formation, cells begin to climb on top of other cells, driven by the formation of new layers and holes at topological defects [22]. The stochastic accumulation of local polar order at $+1/2$ -charge defects gives rise to a local stress accumulation that ultimately extrudes cells out of their original two-dimensional (2D) layer and into a multilayered structure [5]. Cells may alter their own motility in response to chemical signals from other cells or as a starvation response. We aim to investigate whether altering cell motility parameters without invoking explicit chemical signaling, such as cell-cell communication or chemotaxis in a gradient, is sufficient to recapitulate the collective behaviors observed in *M. xanthus* during starvation.

Hydrodynamic modeling of active nematic materials reveals mean-field forces, such as activity, friction, and pressure, which accurately predict the average flows seen in experiments around topological defects [5]. However, hydrodynamic modeling of active nematic materials may miss important features that arise from the discrete nature of the experimental systems, which can be captured by agent-based simulations [23]. We use a bead-spring chain agent-based model to simulate *M. xanthus* colonies and independently tune cell speed and reversal rate. We do not add additional cell signaling or modulate any other aspects of the cell physiology which could occur in experiments that use drugs or mutants to modulate cell motility. Our model captures the collective behaviors observed experimentally in *M. xanthus* while allowing us to independently tune cell speed and reversal period.

II. METHODS

A. Experiment

M. xanthus cells are grown according to standard protocols. Wild-type cells (strain DK1622) are first taken from frozen stocks and struck onto 1.5% agar-CTTYE plates. CTTYE is a nutrient-rich medium, commonly used to culture *M. xanthus*, made of 1% w/v Peptone, 0.5% w/v yeast extract, 10 mM Tris-HCl (pH 8.0), 1 mM KH_2PO_4 , and 8 mM MgSO_4 . Plates are incubated at 32 °C until the first appearance of colonies (about 3 to 4 days). Colonies are then inoculated into liquid media and incubated overnight, shaking at 32 °C. Fresh 1.5% agarose pads filled with rich media are prepared fresh for each experiment and allowed to set before a sample of cells from the overnight liquid culture is spotted onto it. After allowing the spot to dry, this pad is moved to the microscope for imaging.

Cells are imaged by confocal reflected laser light profilometry using a Keyence VK-X1000. This method yields two “images” per field of view: a “reflectance” image where pixel intensity corresponds to the intensity of reflected light measured at the detector, and a “height” image corresponding to the height (dimension axial to the objective) of that pixel. Using this, we are able to view both the cell bodies in the reflectance image, as well as their height relative to the underlying hydrogel surface. Cells are manually segmented and tracked, and simulation parameters are set to match measurements from these tracks. The experimentally measured parameters include cell length, curvature, width, height, speed, and reversal period.

B. Simulation

We model *M. xanthus* cells as chains of beads connected by springs with a harmonic restoring force between connected beads as well as on the angles between neighboring springs to model cell stiffness. We use a truncated Lennard-Jones potential to model repulsive interactions between neighboring cells [Fig. 1(a)]. Our simulations are built within LAMMPS, which was originally created to model equilibrium systems with precise molecular properties [24]. Additional elements to model out-of-equilibrium systems with self-propulsive forces and Brownian dynamics were introduced in Ref. [25]. These propulsion forces are polar, pointing from bead to bead. We introduce custom functions to orient the propulsion dipoles along the spring connecting each bead to the next bead toward the head of the cell so that the self-propulsion of cells acts along the cell body. We use overdamped Brownian dynamics with Euler time integration to model cell motion such that the self-propulsive force drives cells to glide at an intrinsic speed v_0 in the absence of external forces from other cells, which we measure experimentally. Cells reverse their direction of motion with Poisson statistics and an average reversal period of T , which we also measure experimentally. As cells starve and enter their developmental cycle they increase their speed as well as their reversal period. We vary both of these parameters independently to observe their effects on collective motion and the behavior of topological defects. We obtain results for the full phase space over cell speed and reversal period; however, for clarity we present the experimentally observed changes they exhibit during development as a change in Péclet number, Pe . Péclet number represents the ratio of advection to diffusion and can be calculated for reversing self-propelled rods as

$$\text{Pe} = \frac{v_0}{LD_r^{\text{eff}}}, \quad (1)$$

where $D_r^{\text{eff}} = D_r + 2/T_{\text{rev}}$ is the effective rotational diffusion constant accounting for cell reversal events and L is the average cell length [18]. Throughout this paper, we present phase-space plots only for quantities with notable or relevant observations. All remaining phase-space plots are provided in the Supplemental Material (SM) [26].

The aggregation and defect motility effects take place on a much faster timescale than the division and growth rate of *M. xanthus*; therefore, we consider only effects of motility in our simulations and do not include effects that may arise from

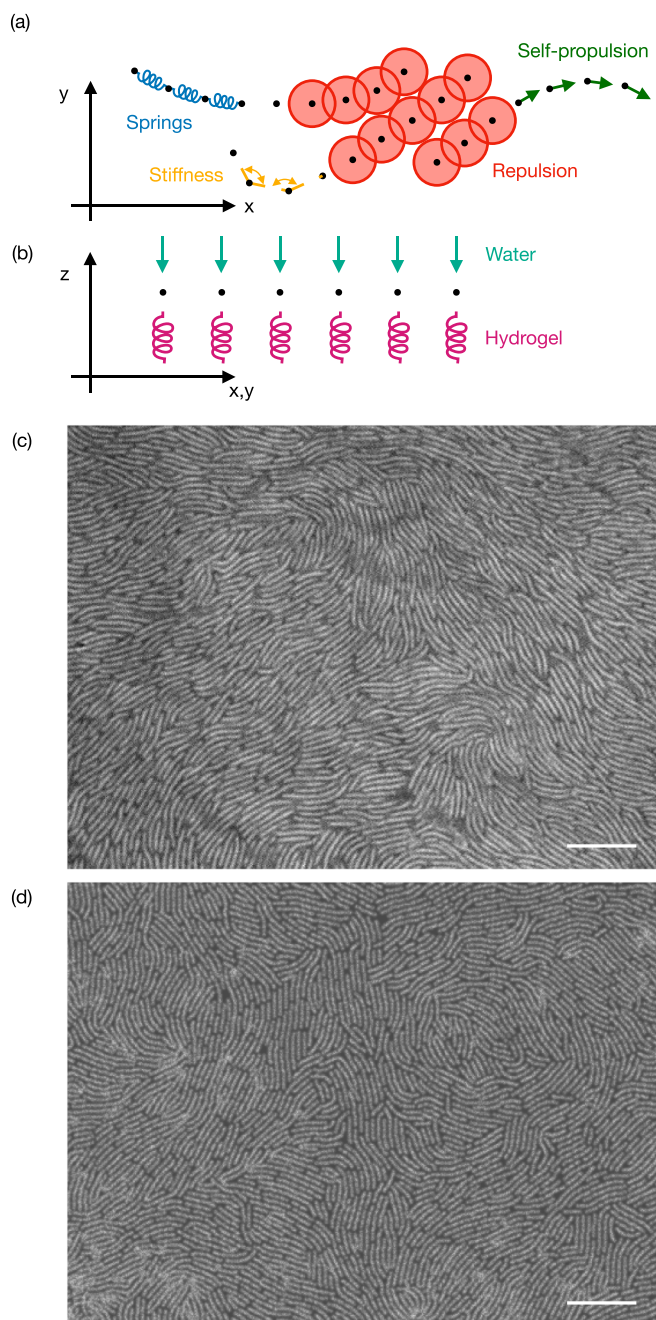


FIG. 1. Setup and overview. (a) Simulation schematic: blue springs connect black bead centers; red denotes truncated Lennard-Jones repulsive interaction, yellow, cell stiffness, and green, self-propulsion. (b) Vertical forces in the simulation: constant downward water force always active (teal), and spring upward force toward $z = 0$ which cuts off above that point (magenta), modeling the hydrogel in experiments. (c) Example experiment image, 10 μm scale bar. (d) Example simulations frame, 10 μm scale bar.

growth. See the SM for details of the simulation as well as experimental measurements of simulation parameters.

All of the forces in the simulations are fully calculated in three dimensions. We apply a soft 2D confinement on the cell layer by modeling the hydrogel as an upward spring that cuts off above $z = 0$, and the layer of water which coats the cells

and holds them on the surface as a constant downward force [Fig. 1(b)]. This constant downward force does not capture all of the effects of the capillary forces that shape the colony edges and promote nematic order through local pressure [27]. However, in the current paper we aim to study the properties of dense continuous monolayers of cells where these edge effects are minimal, and the computational efficiency of modeling the water layer as a constant downward force allows us to model significantly larger systems. An additional benefit of modeling the water forces as a constant downward force is that we are able to randomly initialize very large numbers of cells in a volume above the surface of the gel and allow them to settle from the constant water forces onto the surface. This approach gives us the ability to simulate systems at otherwise inaccessible densities with area packing fractions up to or even above one in a satisfying way. This settling process is similar to what occurs in experiments as the inoculation droplet on a hydrogel dries, leaving the cell layer lying flat on the surface. While this initialization method is a useful tool to allow for modeling very-high-density populations, the details of the initialization do not affect our results as we allow the simulations to run for enough time to reach a steady state before we begin taking data for further analysis.

Most simulation parameters are directly measured in experiments, such as cell length, width, and density. We select cell lengths from a gaussian distribution with a cutoff for minimum and maximum cell lengths. We set the cell bending stiffness in the simulations so that the distribution of cell spline curvatures matches the curvature of cells measured in dense monolayers in the experiments. We finally calibrate the vertical forces in the simulation by measuring a height map of cells on a gel surface using the Keyence profilometer described previously. The height of the top of a cell above the gel surface in simulations is set by the ratio of the gel spring constant and the constant downward water force. We measure this height experimentally and use it to set the ratio of the vertical forces in the simulations. For the majority of this paper we aim to study the quasi-2D active nematic properties of the cell colony, so we limit cell layering while still allowing for small amounts of vertical motion to relieve local pressure allowing for cells to occasionally cross over each other. To achieve this we set relatively high constraining forces in the vertical direction and scale this force with the cell self-propulsive force. Scaling the vertical forces in this way reduces jamming at low cell speeds, allowing them to still relieve local pressure by utilizing the third dimension, while also forcing the high-cellspeed simulations to remain as a monolayer. The amount of cell layering that we observe experimentally varies widely from experiment to experiment likely due to local variations in drying and levels of excreted polysaccharides. Therefore, scaling the vertical forces in our simulations to maintain a monolayer of cells is not inconsistent with the range of vertical forces also present in experiments.

We run simulations for the equivalent of 1 h of experimental time to reach steady state and then analyze simulation results for the following hour, or 6×10^6 time steps. We simulate cells at a density of $0.3 \mu\text{m}^{-2}$ in a periodic box of side length 100 μm . As cells are $5 \pm 1 \mu\text{m}$ long and $0.7 \mu\text{m}$ wide, this is a cell density with a packing fraction of 1 ± 0.1 , as we see in experimental monolayers in high-nutrient conditions

[Fig. 1(c)]. This simulation box size is similar to the field of view for our microscope, which is approximately $100\text{ }\mu\text{m} \times 130\text{ }\mu\text{m}$. Simulated images capture the overall structure of our experimental images, including the formation of a monolayer of densely packed cells with a high degree of local nematic order and pointlike topological defects [Fig. 1(d)].

III. RESULTS

A. Varying cell motility

As *M. xanthus* bacteria run out of nutrients and begin to starve, they enter a part of their developmental cycle where cells increase their Péclet number, Pe (the ratio of advection to diffusion) by moving faster and reversing less often [20]. These changes in motility drive a transition from thin layers of cells that spread over a surface to an aggregated state with mounds of cells surrounded by empty space [18]. In our simulations we have independent control of intrinsic cell speed, v_0 , and reversal period, T , without affecting any other cell behaviors. We modulate cell speed by changing the self-propulsion force. Reported cell speeds, referred to here as "intrinsic cell speed" or v_0 , represent the speed at which cells would move in the absence of other external forces, e.g., from surrounding cells in the bulk. We scan over a range for each parameter, covering those displayed by wild-type cells naturally during starvation, and measure a variety of properties over this phase space. Figure 2(a) shows the changing Péclet number with changing v_0 and T with the experimentally measured cell speed and reversal period outlined in bold having $Pe = 4$, and an arrow showing the experimentally observed increasing Péclet number with development. Future plots that are displayed against Péclet number are measured along this development line, increasing both cell speed and reversal period simultaneously.

We measure the organization of cells within dense monolayers by measuring both the nematic and polar correlation lengths, ξ [Fig. 2(b)]. The polar correlation length reflects the domain sizes over which cells align their self-propulsion direction, while the nematic correlation length reflects the domain sizes over which cells align their long axes with 180° symmetry independent of their head or tail orientation. We find that the nematic correlation length is always greater than the polar correlation length, indicating that the nematic domains are larger than polar domains and suggesting that these cell monolayers form a nematic material. Interestingly, the polar correlation length increases during development while the nematic one remains constant. The buildup of polar order is likely due to the anisotropy in small-angle collisions where nearly parallel rods will increase order while nearly antiparallel ones will leave it unchanged [28]. In our system, reversal events in the polarly ordered domains not only directly reduce polar order but also increase the likelihood of antiparallel interactions. This is the opposite trend of similar modeling with a different mode of propulsion where filaments move by attaching and pushing along neighboring filaments [29].

To examine how cells move within the established nematic structure of these dense monolayers, we measured the mean-square displacement (MSD) of cells within the system at $Pe = 4$ [Fig. 2(c)]. Plotting the MSD on a log-log plot and fitting a

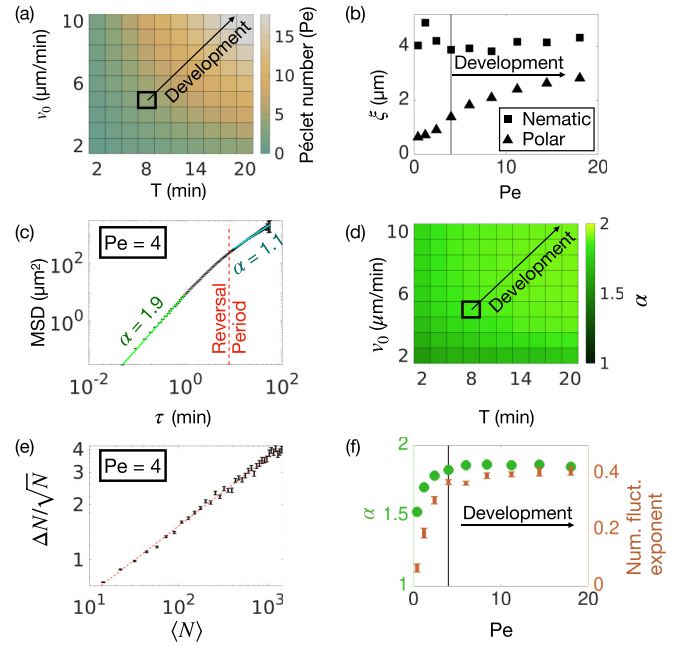


FIG. 2. (a) Péclet number, Pe , over a range of intrinsic cell speeds, v_0 , and reversal periods, T . The value corresponding to experimentally v_0 and T is outlined in bold and an arrow shows the direction of motility changes displayed by *M. xanthus* during starvation. (b) Polar and nematic correlation lengths, ξ plotted against Pe . The vertical line shows $Pe = 4$ parameters with the arrow showing the changes made by *M. xanthus* during development. Error bars are the 95% confidence interval for the fit and are smaller than the displayed points. (c) Mean-square displacement of individual cells within the dense monolayer at $Pe = 4$. The green line indicates the fit line for small timescales with $\alpha = 1.9$, while the cyan line shows the fit line for long timescales with $\alpha = 1.1$. The reversal period is indicated by the red line. Error bars are standard error. (d) α for the short timescale fits ($\tau < 1$ min) for cells within a dense monolayer in simulations over a range of v_0 and T . (e) Standard deviation of particle number measured for increasing region sizes in simulations at experimentally matched conditions with $Pe = 4$, plotted against average particle number. Error bars are the standard error; dashed line is the fit to the data. (f) Exponent of number fluctuations calculated from the fit illustrated in (e) plotted against Pe during development (orange) along with the short-timescale α values extracted from the phase space in (d) (green). Error bars are the 95% confidence interval for the fit lines, and are smaller than the plotted points for α values.

line, we can extract the diffusivity exponent, α , as the slope of the fitted line in log-log space. We find that at short timescales α is close to 2, suggesting that cells move ballistically and are able to glide past each other uninhibited. At timescales above the reversal period this changes to a value of α close to 1, which represents purely diffusive motion, and is the same trend observed previously in low-density reversing self-propelled rods [8]. We measure α at small timescales ($\tau < 1$ min) over our phase space to examine where cell motion is uninhibited [Fig. 2(d)] and find that cells move ballistically at short timescales ($\alpha \approx 2$) for the majority of the phase space. However, for low v_0 , the small-timescale α 's drop significantly, indicating that cell motion is inhibited in this regime. This inhibition is likely due to the decreased average active

force in this regime causing cells to be unable to overcome the vertical confining forces and release local pressure, something reminiscent of a jamming transition (see SM Sec. II).

As an active material we expect our system to display giant number fluctuations [30]. We measure this by breaking the system into smaller boxes of different sizes, and measure the average ($\langle N \rangle$) and standard deviation (ΔN) of the number of beads in the all boxes of increasing sizes. Normalizing ΔN by $\sqrt{N}$ and plotting it on a log-log scale against $\langle N \rangle$ results in an exponent of 0.5 for an active material and 0 for a passive one, and indeed we observe giant number fluctuations with an exponent of 0.5 as expected for an active material at our experimentally measured parameters [Fig. 2(e)] [30]. During development, with increasing Péclet number, cell motion remains uninhibited and the colony forms an active material as seen by α remaining close to 2 and the number fluctuations exponent close to 0.5. However, interestingly, for very small Péclet numbers the cell motion is inhibited and the system forms something more like a passive nematic liquid crystal [Fig. 2(f)]. Having found that our cells layers form an active nematic material, we set out to measure defect properties within the cell monolayers during development.

B. Topological defects

Topological defects are points in a nematic material where the local molecular alignment breaks down, the orientation is undefined, and the topological winding number is nonzero. Topological defects have been shown to be an important feature of active nematic materials. In the absence of topological defects particles are well aligned and local active stresses cancel out. However, in an extensible active nematic material, local bend deformations in particle alignment are unstable and grow, leading to the formation of pairs of oppositely charged defects [31]. These topological defects provide sites where the local active stress drives flows of material, leading to comet-shaped $+1/2$ defects self-propelling while the threefold-symmetric $-1/2$ defects diffuse passively [32]. The self-propelling $+1/2$ defects can provide a mechanism for mixing within active nematic materials [33] and the flows of many defects combine to cause the material flows to form vortices and exhibit turbulence [34].

We identify topological defects by interpolating the bead dipoles, which point along the cell bodies, to find the orientation of the director field (θ). We then calculate the winding number ($q = \int_0^{2\pi} d\theta$) on a grid at every vertex of four interpolated grid points. We then combine all charged points within a minimum distance of each other, $r_{\min} = 1.4 \mu\text{m}$ (two cell widths), by summing their charges and averaging their locations in order to filter out transient local charge pairs that arise due to small fluctuations in bead orientations and noise. This approach also allows us to detect any integer charge defects if they should arise, though we very rarely if ever detect them in our simulations or experiments. We observe $\pm 1/2$ defects in our simulations which closely resemble those seen in experiments. The examples in Fig. 3(a) (experiments) and Fig. 3(b) (simulations) show a cell image overlaid with local cell orientation, or director field (θ), along with a defect pair with the $+1/2$ defect displayed as a red comet shape, and the $-1/2$ defect as a blue triangle.

Next we measure the average flow field around each type of defect. In the experiments we use the Farneback algorithm to measure optical flow across the whole field of view at all times, while the simulations simply output the velocity vector for each bead at each time point. From these individual velocity flow fields we extract and reorient the local velocity field around each defect so that all defects are centered and aligned. We then average together the flow fields for each charge of defect to obtain a mean velocity field around $+1/2$ defects in experiments [Fig. 3(c)] and simulations [Fig. 3(d)], as well as around $-1/2$ defects in both experiments [Fig. 3(f)] and simulations [Fig. 3(g)]. The flow fields around defects in simulations share the same structure and are very similar even in magnitude to those measured in experiments despite the fact that the individual cell speeds are nearly five times higher ($\approx 5 \mu\text{m}/\text{min}$) than the peak speed in the average flow fields ($\approx 1 \mu\text{m}/\text{min}$), confirming that the simulations are capturing the basic mechanisms and mean-field defect behaviors observed in experiments quantitatively as well as qualitatively. We observe a weak chirality in the experimental flow field [Fig. 3(d)], likely arising from the molecular motility mechanism, in which molecular motors are thought to move along an internal helical protein filament [35]. This chirality appears to emerge at the collective scale, and understanding its implications for collective cell motion is an intriguing direction for future study.

Cell aggregation which leads to fruiting-body formation is driven at this intermediate length scale by the accumulation of cells at $+1/2$ defects and depletion from $-1/2$ defects [22]. We measure the amount of accumulation or depletion taking place by measuring the net flow through a circle around each type of defect at a radius (R) which maximizes the magnitude [$V_{\text{net}}(R) = -1/2\pi \int_0^{2\pi} v_r(R, \phi) d\phi$, where $v_r(R, \phi) = \vec{v}(R, \phi) \cdot \vec{R}/R$]. Figures 3(c) and 3(f) show a schematic taken from the simulation flow fields, illustrating the flow through a circle around the center of $+1/2$ and $-1/2$ defects, respectively; the net flow is the sum of the radial component of these flows, with the net flow plotted in Fig. 3 taken at the radius which maximizes the magnitude of this quantity. Finally, we calculate the net flow around positive and negative defects against Péclet number and find that the magnitude of the net flow around both charges of defects steadily increases with development. The negative values for $-1/2$ defects reflects a net outflow while the positive values represent a net inflow seen at $+1/2$ defects. Knowing that the average inflow or outflow increases around defects with development, we next measure what the defects themselves are doing.

C. Defect dynamics

As cell accumulation and depletion occur at topological defects, not only does the net flow affect this accumulation process, but defect density and motility also likely play a role. We expect defects which remain stationary for longer to allow for greater accumulation at single locations over time, compared to defects which are too motile [5]. To measure defect motility, we track defects over time by simple nearest-neighbor tracking between frames while conserving charge, and use these defect tracks to calculate the mean-square displacement over time with $\text{Pe} = 4$ [Fig. 4(a)]. As expected

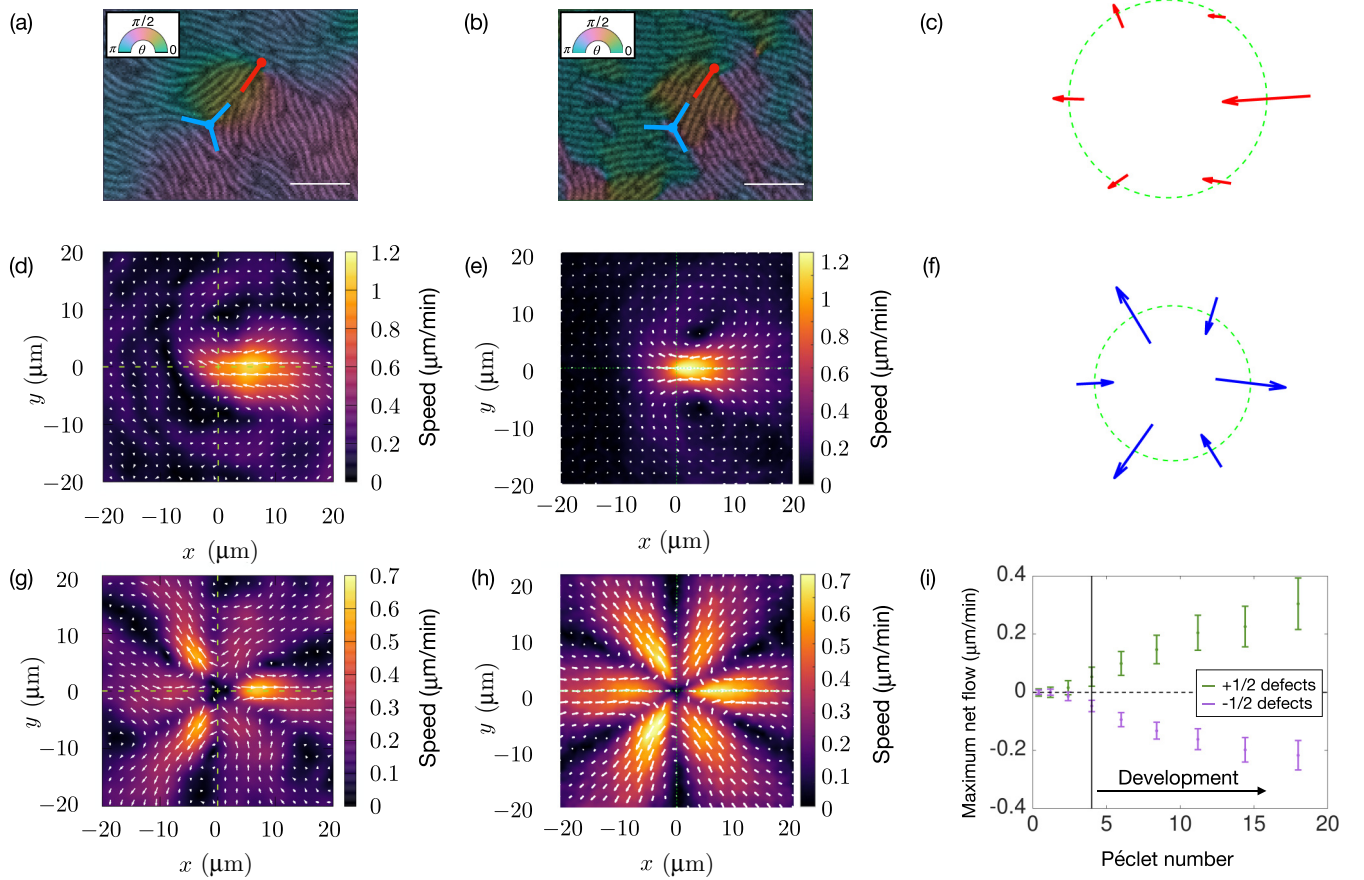


FIG. 3. Defects and flows. (a) A pair of defects from an experimental image overlaid with color representing the local director, 5 μm scale bar. (b) A pair of defects from simulations also colored by local director, 5 μm scale bar. Positive defects are illustrated in red, and negative in blue. [(d), (g)] Experimental flow fields averaged over all positive and negative defects, respectively. [(e), (h)] Simulated flow fields averaged over all positive and negative defects, respectively. [(c), (f)] Illustration of the velocity vectors at the radius where the magnitude of the net flow is maximized for positive and negative defects, respectively; the net flow is calculated as the sum of the radial components of the displayed vectors. (i) The maximum net flow at positive and negative defects measured against Pe from the simulation data.

for an active nematic material, where $+1/2$ defects self-propel, the slope of the MSD on a log-log plot for $+1/2$ defects is greater than that of the $-1/2$ defects, which are expected to be more diffusive [32]. We then fit the MSD for $+1/2$ and $-1/2$ defects and measure α vs Pe during development and find that α is always higher for the $+1/2$ defects than the $-1/2$ ones, and both remain constant or perhaps very

slightly increasing [Fig. 4(b)]. This finding suggests that during development defect motility is mostly constant. However, at very low Pe number where the bacteria colony appears more like a passive nematic material than an active one, α drops to something close to 1 and is roughly equal for both positive and negative defects, as expected in a passive nematic liquid crystal.

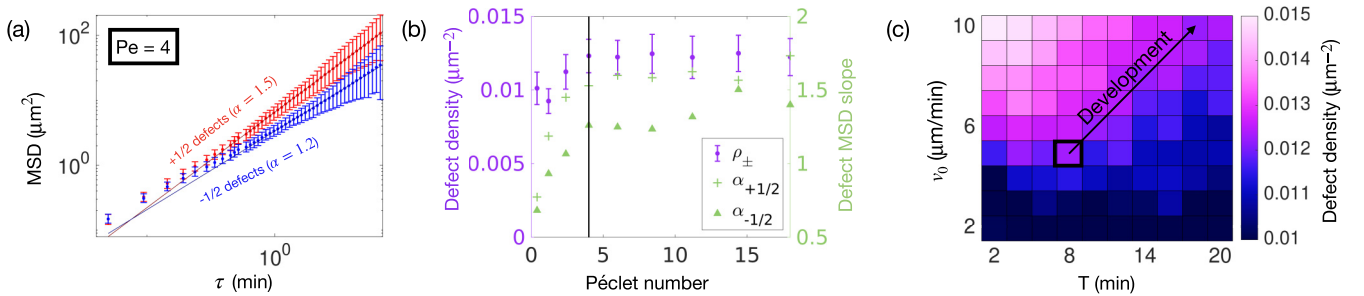


FIG. 4. Defect behaviors. (a) Mean-square displacement of positive (red) and negative (blue) defects for experiment matched conditions. (b) Defect density against Pe with standard error as the error bars (purple, left axis). Fit exponent (α) for positive (plus signs) and negative (triangles) defects plotted against Pe during development (green, right axis). The error bars as the 95% confidence interval from the fit are smaller than the width of the line. (c) Phase space of defect density against both v_0 and T . Experimentally matched conditions are outlined in bold and an arrow represents changes displayed by cells during development.

We then measure the average defect density across all frames and simulations to examine how it changes with development. We find that defect density remains constant with increasing Pe during development [Fig. 4(b)]. Interestingly, if we examine defect density across the entire phase space of v_0 vs T we find that the relationship of defect density with Pe is not as simple as it might first appear [Fig. 4(c)]. The fact that cells increase their Péclet number by changing both speed and reversal period simultaneously is necessary for the defect density to remain constant. If cells were to only increase their reversal period without also increasing cell speed the defect density would decrease. Conversely, if cells increased speed without increasing reversal period the defect density would actually increase. Together these results reveal that, as cells increase their Péclet number by increasing both their speed and reversal period during development, they maintain a constant nematic and defect structure within the cell colony.

D. Cell aggregation

As cells speed up and reverse less often the colony aggregates and begins to pile up, causing it to have regions with two layers of cells as well as areas with no cells at all. This presents challenges for accurately simulating the cell layers as the details of the interactions between the first and second layer of cells is unclear. Cell gliding is driven by motors which form focal adhesions with the surface they are gliding across and how these gliding forces are transmitted when cells are gliding on top of other cells is unclear [36]. To avoid these complications in the previous sections of this paper we scale the vertical confinement forces with the active force in order to prevent layering. In order to see whether the aggregation in our simulations is consistent with that observed experimentally, we now fix the vertical confining forces to an intermediate value while varying only cell speed and reversal period. Figure 5(a) shows an example simulation output image at our experimentally measured condition in high nutrients, with $Pe = 4$, where we expect cells to spread out to cover the surface, and indeed we see that they do. Figure 5(b) shows an example image at $Pe = 18$ with higher speed and reversal rate as seen in experimental systems when cells begin to aggregate into fruiting bodies during starvation. We see a similar aggregation behavior in our simulations with both an increase in empty areas as well as regions with two layers of cells.

We quantify the amount of aggregation and layering occurring by simply looking at the z positions of the simulated cells. Figure 5(c) shows a histogram of the z position of beads for the two images shown in Figs. 5(a) and 5(b). We can see that for the low- Pe conditions (red) there is a single central peak in the histogram at a value of zero illustrating that the cells form a single monolayer in these conditions. However, at high Pe we see this same central peak as well as two side peaks. The central peak is from the regions of the simulations where a monolayer exists, while the two side peaks are from regions with two layers of cells. Since the hydrogel is modeled as a simple harmonic spring force, when cells climb into the second layer they also push the first layer of cells down into the gel, resulting in a value of $z \approx 0.3$ for cells in the second layer, and $z \approx -0.3$ for cells in the layer below them. These height values are consistent with cells of a radius of $0.7 \mu\text{m}$

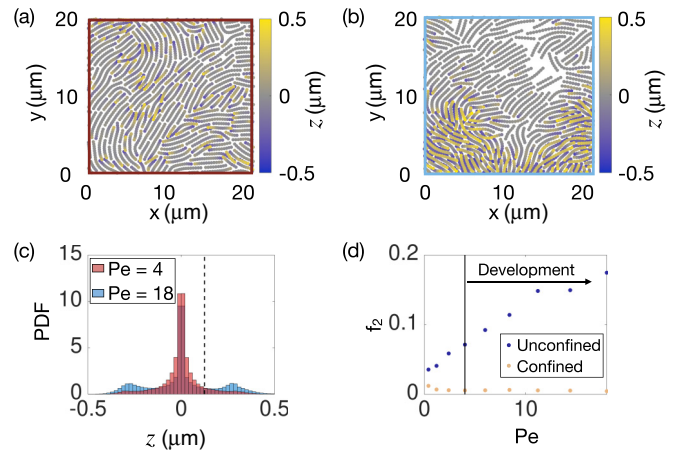


FIG. 5. Aggregation. [(a), (b)] Example simulation output colored by bead z position. (a) Parameters are set to the experimentally measured high-nutrient conditions with $Pe = 4$ where a monolayer is expected. (b) Parameters are set to high speed and reversal period with $Pe = 18$ where aggregation and increased layering is expected. (c) Histogram of bead z positions for the two examples shown in (a), $Pe = 4$, and (b), $Pe = 18$. The dashed vertical line shows the height threshold between the bottom and top layers of cells. (d) The fraction of cells in the second layer plotted against Pe during development. The peach points show the simulations where the vertical z forces are scaled with active force in order to prevent layering and maintain a monolayer of cells as presented in the previous sections of this paper. The blue points show the values when the vertical forces are held constant at an intermediate value while only cell speed and reversal period are varied. Error bars are the standard error and are smaller than the displayed points.

interweaving within the grooves between adjacent cells in the bottom layer. This process of cells climbing up into a second layer as well as their being pushed down into the gel are both consistent with previous experimental observations where second layers of cells on stiff gel substrate emerge above the bottom monolayer of cells [22], while cells aggregating on a very soft gel actually form the second layer downward within the gel as this presents less resistance than deforming the water layer that coats the colony [5,27].

To examine this aggregation trend with increasing Pe during development we measure the fraction of beads with $z > 0.15 \mu\text{m}$, which are those in the second layer, and plot this fraction against Pe . Figure 5(d) shows this fraction during development when cells are free to pile up (blue), and indeed we see an increase in the fraction of cells in the second layer with increasing Pe , indicating an increase in aggregation during the development process, as expected. To confirm these calculations we also measure the fraction of cells in the second layer for the simulations from the previous sections where cells are prevented from climbing into the second layer in Fig. 5(d) (peach). Indeed, in these conditions we see that cells are unable to pile up or aggregate, as we designed it to be.

IV. DISCUSSION

Myxococcus xanthus is a soil bacterium with a developmental cycle where large collectives of hundreds of thousands

to millions of cells transition from a thin spread-out sheet of cells and aggregate into 3D droplets of cells separated by empty space. We know that this aggregation process is driven by individual cells tuning their motility to increase Péclet number (Pe) by speeding up and reversing less often [18]. We also know that on an intermediate length scale cells tend to align their long axes within a local neighborhood and the 2D-to-3D transition is initiated by layering events at topological defects where cells are misaligned [22]. Here, we present a simulation of *M. xanthus* as bead-spring chains where we can investigate the properties of topological defects as cells increase their Pe.

We find that modeling *M. xanthus* populations as a dense collection of flexible self-propelled filaments, or bead-spring chains, captures the macroscopic monolayer structures observed in experimental systems with the same topological defects present. Varying the Pe in simulations by increasing both cell speed and reversal period drives the system into an aggregated state just as the cells do naturally in experiments. Looking closer at the cell structure within the system with increasing Pe we find that, first, the nematic correlation length is always longer than the polar one, suggesting that we have a nematic material. While the nematic correlation length remains constant with Pe, the polar correlation length actually increases with increasing Pe, suggesting that any effects driven by instantaneous cell polarity may increase during development.

Within the nematic structure of the cell monolayers, cells are able to glide past each other uninhibited by their surroundings, as indicated by the short-timescale α 's of cell mean-square displacements remaining close to 2. The number fluctuations scale with mean particle number with an exponent greater than zero, confirming that we have an active nematic material. However, at very small Pe both α and the number fluctuations exponent drop significantly, suggesting that when cell motion is inhibited by surrounding cells in a dense monolayer, the material begins to behave more like a passive nematic material than an active one.

When we measure the flow around topological defects during development we find that there is an increased inflow at positive defects with increasing Pe, and a corresponding increased outflow at negative defects, which drives aggregation during starvation. Investigating the motion of defects and their density reveals that these quantities do not change significantly during development. Interestingly, the constant defect density relies on cells modulating their Pe by changing both speed and reversal period simultaneously. Were cells to

only increase speed or reversal period during starvation and not both, the defect density would also change with starvation rather than remaining constant.

In this paper we show that a dense bead-spring chain model captures the collective behaviors of *M. xanthus* bacteria colonies. As cells starve and increase their Pe they maintain an unchanging nematic structure while increasing the net flow around topological defects driving the aggregation process. This increased net flow may be tied to an increasing polarity we also observe with increasing Pe. In the present study we are unable to model the full effects of the water layer coating the cells which effectively smoothes out the edges of holes and new layers as they form. This effect will have implications for the cells as they aggregate into larger and larger mounds and is a topic for future study. The question of why a constant defect density may be advantageous during starvation is unclear and a topic for future study, as is the long-term aggregation process connecting this initial layering to the full formation of relatively large 3D droplets. Increasing the flow at defects is typically driven by increasing the mean-field activity in an active nematic material, which can also increase defect number and defect motility. In order to build large aggregates, it is likely that cells must maintain a relatively low number of defects with limited mobility, thereby preventing the continual formation and dissolution of many small layers. It seems that *M. xanthus* has found an elegant solution to this potential problem by increasing the flow at defects and therefore aggregation by changing both cell speed and reversal period.

ACKNOWLEDGMENTS

We thank N. Wingreen, C. Fei, A. Bourque, B. Figueiredo, A. Al Harraq, R. Alert, and A. Pyo for discussions. This work was supported in part by the National Science Foundation, through Award No. PHY-1806501 and the Center for the Physics of Biological Function.

K.C. designed and ran simulations. K.C. and M.B. performed and analyzed experiments. J.W.S. supervised the study. All authors interpreted data and results. K.C. wrote the manuscript with input from all authors.

There are no conflicts to declare.

DATA AVAILABILITY

The data that support the findings of this article are openly available [37].

-
- [1] M. Bär, R. Großmann, S. Heidenreich, and F. Peruani, Self-propelled rods: Insights and perspectives for active matter, *Annu. Rev. Condens. Matter Phys.* **11**, 441 (2020).
 - [2] A. Sciortino and A. R. Bausch, Pattern formation and polarity sorting of driven actin filaments on lipid membranes, *Proc. Natl. Acad. Sci. USA* **118**, e2017047118 (2021).
 - [3] A. Sokolov and I. S. Aranson, Physical properties of collective motion in suspensions of bacteria, *Phys. Rev. Lett.* **109**, 248109 (2012).
 - [4] L. Huber, T. Krüger, and E. Frey, Microphase separation in active filament systems maintained by cyclic dynamics of cluster size and order, *Phys. Rev. Res.* **3**, 013280 (2021).
 - [5] E. Han, C. Fei, R. Alert, K. Copenhagen, M. D. Koch, N. S. Wingreen, and J. W. Shaevitz, Local polar order controls mechanical stress and triggers layer formation in *myxococcus xanthus* colonies, *Nat. Commun.* **16**, 952 (2025).

- [6] R. Großmann, F. Peruani, and M. Bär, Diffusion properties of active particles with directional reversal, *New J. Phys.* **18**, 043009 (2016).
- [7] J. Taktikos, H. Stark, and V. Zaburdaev, How the motility pattern of bacteria affects their dispersal and chemotaxis, *PLoS One* **8**, e81936 (2013).
- [8] I. Santra, U. Basu, and S. Sabhapandit, Active brownian motion with directional reversals, *Phys. Rev. E* **104**, L012601 (2021).
- [9] K. Kawaguchi, R. Kageyama, and M. Sano, Topological defects control collective dynamics in neural progenitor cell cultures, *Nature (London)* **545**, 327 (2017).
- [10] J. E. Johansen, J. Pinhassi, N. Blackburn, U. L. Zweifel, and Å. Hagström, Variability in motility characteristics among marine bacteria, *Aquat. Microb. Ecol.* **28**, 229 (2002).
- [11] G. M. Barbara and J. G. Mitchell, Bacterial tracking of motile algae, *FEMS microbiol. ecol.* **44**, 79 (2003).
- [12] C. Tamulonis, M. Postma, and J. Kaandorp, Modeling filamentous cyanobacteria reveals the advantages of long and fast trichomes for optimizing light exposure, *PLoS One* **6**, e22084 (2011).
- [13] A. Be'er, S. K. Strain, R. A. Hernández, E. Ben-Jacob, and E.-L. Florin, Periodic reversals in *paenibacillus dendritiformis* swarming, *J. Bacteriol.* **195**, 2709 (2013).
- [14] M. J. Kühn, L. Talà, Y. F. Inclan, R. Patino, X. Pierrat, I. Vos, Z. Al-Mayyah, H. Macmillan, J. Negrete, Jr., J. N. Engel *et al.*, Mechanotaxis directs *pseudomonas aeruginosa* twitching motility, *Proc. Natl. Acad. Sci. USA* **118**, e2101759118 (2021).
- [15] U. Pfreundt, J. Słomka, G. Schneider, A. Sengupta, F. Carrara, V. Fernandez, M. Ackermann, and R. Stocker, Controlled motility in the cyanobacterium *trichodesmium* regulates aggregate architecture, *Science* **380**, 830 (2023).
- [16] D. Kaiser and R. Welch, Dynamics of fruiting body morphogenesis, *J. Bacteriol.* **186**, 919 (2004).
- [17] J. Muñoz-Dorado, F. J. Marcos-Torres, E. García-Bravo, A. Moraleda-Muñoz, and J. Pérez, Myxobacteria: Moving, killing, feeding, and surviving together, *Front. microbiol.* **7**, 781 (2016).
- [18] G. Liu, A. Patch, F. Bahar, D. Yllanes, R. D. Welch, M. C. Marchetti, S. Thutupalli, and J. W. Shaevitz, Self-driven phase transitions drive *myxococcus xanthus* fruiting body formation, *Phys. Rev. Lett.* **122**, 248102 (2019).
- [19] L. Jelsbak and L. Søgaard-Andersen, Pattern formation by a cell surface-associated morphogen in *myxococcus xanthus*, *Proc. Natl. Acad. Sci. USA* **99**, 2032 (2002).
- [20] R. Mercier and T. Mignot, Regulations governing the multicellular lifestyle of *myxococcus xanthus*, *Curr. Opin. Microbiol.* **34**, 104 (2016).
- [21] T. Sanchez, D. T. Chen, S. J. DeCamp, M. Heymann, and Z. Dogic, Spontaneous motion in hierarchically assembled active matter, *Nature (London)* **491**, 431 (2012).
- [22] K. Copenhagen, R. Alert, N. S. Wingreen, and J. W. Shaevitz, Topological defects promote layer formation in *myxococcus xanthus* colonies, *Nat. Phys.* **17**, 211 (2021).
- [23] M. Dedenon, C. Blanch-Mercader, K. Kruse, and J. Elgeti, The importance of being discrete—an agent-based model for active nematics and more, *arXiv:2501.04559*.
- [24] A. P. Thompson, H. M. Aktulga, R. Berger, D. S. Bolintineanu, W. M. Brown, P. S. Crozier, P. J. In't Veld, A. Kohlmeyer, S. G. Moore, T. D. Nguyen *et al.*, lammps—a flexible simulation tool for particle-based materials modeling at the atomic, meso, and continuum scales, *Comput. Phys. Commun.* **271**, 108171 (2022).
- [25] R. G. Winkler, A. Wysocki, and G. Gompper, Virial pressure in systems of spherical active brownian particles, *Soft Matter* **11**, 6680 (2015).
- [26] See Supplemental Material at <http://link.aps.org/supplemental/10.1103/jfjqj-2n4q> for supplemental figures and methods.
- [27] M. E. Black, C. Fei, R. Alert, N. S. Wingreen, and J. W. Shaevitz, Capillary interactions drive the self-organization of bacterial colonies, *Nat. Phys.* **21**, 1444 (2025).
- [28] A. Baskaran and M. C. Marchetti, Self-regulation in self-propelled nematic fluids, *Eur. Phys. J. E* **35**, 95 (2012).
- [29] G. A. Vliegthart, A. Ravichandran, M. Ripoll, T. Auth, and G. Gompper, Filamentous active matter: Band formation, bending, buckling, and defects, *Sci. Adv.* **6**, eaaw9975 (2020).
- [30] H. Chaté, F. Ginelli, and R. Montagne, Simple model for active nematics: Quasi-long-range order and giant fluctuations, *Phys. Rev. Lett.* **96**, 180602 (2006).
- [31] M. C. Marchetti, J.-F. Joanny, S. Ramaswamy, T. B. Liverpool, J. Prost, M. Rao, and R. A. Simha, Hydrodynamics of soft active matter, *Rev. Mod. Phys.* **85**, 1143 (2013).
- [32] L. Giomi, M. J. Bowick, P. Mishra, R. Sknepnek, and M. Cristina Marchetti, Defect dynamics in active nematics, *Philos. Trans. R. Soc. A* **372**, 20130365 (2014).
- [33] A. J. Tan, E. Roberts, S. A. Smith, U. A. Olvera, J. Arteaga, S. Fortini, K. A. Mitchell, and L. S. Hirst, Topological chaos in active nematics, *Nat. Phys.* **15**, 1033 (2019).
- [34] L. Giomi, Geometry and topology of turbulence in active nematics, *Phys. Rev. X* **5**, 031003 (2015).
- [35] B. Nan, J. Chen, J. C. Neu, R. M. Berry, G. Oster, and D. R. Zusman, Myxobacteria gliding motility requires cytoskeleton rotation powered by proton motive force, *Proc. Natl. Acad. Sci. USA* **108**, 2498 (2011).
- [36] R. Balagam, D. B. Litwin, F. Czerwinski, M. Sun, H. B. Kaplan, J. W. Shaevitz, and O. A. Igoshin, *Myxococcus xanthus* gliding motors are elastically coupled to the substrate as predicted by the focal adhesion model of gliding motility, *PLoS Comput. Biol.* **10**, e1003619 (2014).
- [37] K. Copenhagen, Myxo simulations, <https://github.com/kcopenhagen/lammps-myxosim>