A motility-induced phase transition drives *Myxococcus xanthus* aggregation

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A hallmark of living systems is their ability to generate complex spatial patterns at the molecular, cellular, and multicellular levels. Many such systems rely on coupled biochemical and genetic signaling mechanisms that can produce large-scale organization. Long-range order and patterning can also emerge, however, through purely mechanical interactions. Here, we study the starvation-induced aggregation of gliding *Myxococcus xanthus* bacteria and show that these cells phase separate by tuning their motility over time. By experimentally varying the density and speed of gliding cells, tracking individual cells in large populations, and comparing to simulations of a model of reversing Active Brownian Particles (ABPs), we show that cell aggregation can be understood with a single phase diagram in terms of density and a dimensionless inverse rotational Péclet number that characterizes cell motility. We further track changes in motility of the wild-type during starvation and show that a reduction of the reversal frequency and an increase in gliding speed change the rotational Péclet number to drive aggregation. Thus, *M. xanthus* evolved to take advantage of an active-matter phase transition that can be controlled through changes in motility at the individual cell level without complex feedback and chemical communication between cells.

I. INTRODUCTION

Unicellular organisms such as bacteria or amoeba are sometimes capable of spontaneously organizing into complex multicellular structures [1,2]. A striking example of such collective behavior is the starvation-induced organization of the rod-shaped, soil-dwelling bacterium *Myxococcus xanthus* into three-dimensional multicellular aggregates known as “fruiting bodies” [3]. When nutrients are readily available, a swarm of *M. xanthus* cells expands to prey on other bacterial species by secreting antibiotics and lytic enzymes [4,5]. When nutrients are scarce, however, starving bacteria undergo a multicellular process of self-organization during which cells move to form dome-shaped aggregates comprising hundreds of thousands of cells. A subset of cells at the center of each aggregate differentiate to form metabolically quiescent spores that can survive long periods of starvation [3,6,7].

Superficially, the striking phenotypic similarity between aggregation in the amoeba *Dictyostelium discoideum* and *M. xanthus* has led to the longstanding hypothesis that *Myxococcus* fruiting body formation is driven by chemical signaling mechanisms as it is in the amoeba. However, despite decades of research, the links between chemical signaling and the direct mechanisms of aggregation remain unclear. Although *M. xanthus* cells are known to employ chemical communication to initiate aggregation (termed A-signaling) [8], to potentially synchronize reversal frequency (termed C-signaling) [9,10], and to communicate through the production of mucus polysaccharide “slime trails” that other cells can sense and follow [11], a quantitative understanding of the mechanisms that drive aggregation has remained elusive.

Both the biochemical and mechanical aspects of multicellular organization are driven by the dissipation of chemical energy keeping the system far from equilibrium. Efforts to understand collective behavior in far from equilibrium systems from generic interactions between individuals in large ensembles have led to statistical approaches predicting broadly applicable organizational principles, phase transitions, and scaling laws [12–14]. In the past decade, hydrodynamic theories have been developed [12] to understand the large-scale, long-time behavior of a wide class of active systems, defined as out of equilibrium systems comprised of individual units each consuming and dissipating energy. Active matter theories [15] have been successfully applied to such diverse phenomena as bird and animal flocks [16], tissue-level changes during embryogenesis [16], collective cell migration in wound healing [17,18], pattern formation in motile microorganisms [19], the assembly of the mitotic spindle [20], and synthetic active systems [21].

Perhaps the most remarkable properties of systems comprised of self-propelled units are their ability to spontaneously generate flows [15] and to phase separate into dense and dilute phases even in the absence of attractive interactions [21]. As a consequence of the breaking of detailed balance at the microscale, populations of motile individuals can undergo spontaneous aggregation without
the need for additional signaling mechanisms or cohesive forces between individual units [22].

Phase separation arises when the time for self-propelled units to reorient their direction of motion after a collision exceeds the mean free time between collisions, giving rise to the formation of long-lived clusters of particles. This phenomenon, which does not have a counterpart in passive systems where the constituents move via random thermal excitations, has been termed motility-induced phase separation (MIPS) [21, 23–26]. The dynamics of the phase separation process is controlled by the initial density of the units and can be quantified in terms of the non-dimensional inverse rotational Péclet number, the ratio of particle size $\ell_p$ to the persistence length $\ell_p$,

$$ Pe_{r}^{-1} = \ell_p / \ell_c = \ell_c D_r / v_0, \quad (1) $$

where $v_0$ is the self-propulsion speed and $D_r$ is the rotational diffusion coefficient. Starting from a homogeneous dilute system, increasing density or decreasing the inverse Péclet number will favor first the nucleation of short-lived clusters and then spontaneous spinodal decomposition into bulk phase separated gas and condensed states. Data obtained in systems of varying motility and rotational diffusion rate can be organized into a phase diagram controlled entirely by $Pe_{r}^{-1}$ and the density.

By examining the spatial density profile of aggregating cells combined with single-cell tracking and active-particle simulation, we show that the dynamical mechanism underlying $M. xanthus$ aggregation is a MIPS process. At low cell density, the aggregation occurs via a nucleation-and-growth process whereby small aggregates appear asynchronously and grow steadily as development proceeds. At high cell density, the dynamics resemble a spontaneous spinodal-like process, with phase separation occurring everywhere at once, resulting in a connected network of allantoid aggregates that then resolve into circular aggregates during development (Fig.1). We control the motility properties of gliding cells using a mutant strain and chemical perturbations to show that when scaled in terms of an effective Péclet number constructed by independent measures of $M. xanthus$ motility parameters, the experimental data can be organized into a phase diagram that shows striking quantitative correspondence to that of active Brownian particles. Additionally, the observed kinetics of aggregate growth agree quantitatively with that of MIPS coarsening. We conclude that, when starved, $M. xanthus$ cells dynamically regulate their motility properties to traverse phase space and position the population in a region of low effective inverse Péclet number where spontaneous phase separation occurs.

FIG. 1. Aggregation in $M. xanthus$ (A-B) and in simulations of ABPs (C-D). The snapshots for both systems are taken at equal times in terms of the single-particle reorientation time ($\tau_r \approx 10$ min for the bacteria). If the density is sufficiently low, no aggregation occurs. Nucleation and growth: we compare $M. xanthus$ at a concentration of $3 \times 10^5$ cell/ml (A) and simulations of ABPs with inverse Péclet number $Pe_{r}^{-1}=0.01$ and packing fraction $\phi = 0.29$ (C). In both cases, small clusters form at random times and positions, only some of which develop into large nucleation centers. Spinodal decomposition: for larger densities, particle aggregates form everywhere at the same time and coarsen. This is shown for $M. xanthus$ at a concentration of $1 \times 10^6$ cell/ml (B) and for ABPs with $Pe_{r}^{-1}=0.01$ and $\phi = 0.5$ (D). Simulation snapshots have a linear size $L = 1000a$.

II. RESULTS

A. Reversing Active Brownian Particles

$M. xanthus$ cells move by gliding on solid surfaces using both tank-tread-like transport motors and the retraction of extruded filaments called pili. It has been shown that these cells can modulate their speed in a seemingly continuous manner [27–28]. While cells do not appear to have the ability to steer in two dimensions during gliding, they are able to reverse the direction of all the transport motors quickly, causing them to move in the opposite direction. These “reversals” typically occur every several minutes, and cells modify the reversal frequency in different situations [29–31].

Previous numerical work on reversing active particles
FIG. 2. Modeling *M. xanthus* cells as reversing active Brownian particles. (A) Schematic of our minimal ABP model. A single particle has radius $a$ and moves with velocity $v_0 \hat{n}_i$. The direction of self-propulsion is continuously affected by a white noise with variance proportional to $D_r$ and directional reversals at times given by a Poisson process with frequency $f_{\text{rev}}$. These two parameters can be combined into an effective rotational diffusion coefficient: $D_{\text{eff}} = D_r + 2 f_{\text{rev}}$. In addition, there is a spring-like repulsive interaction force $F_{ij}$ between each pair of particles. High-density clusters nucleate when colliding particles are caged by their surrounding neighbors before being able to reorient. (B) Mean square displacement (MSD) versus time for single particles, plotted for various reversal frequencies collapse when time is scaled by the reorientation time $\tau_r = D_{\text{eff}}$.

The dynamical mechanism of aggregation depends on cell density

We first investigated the effects of different cell densities on *M. xanthus* aggregation. In our experiments, we spotted a drop of bacterial liquid culture of known density onto a minimal media agar substrate (see Materials and Methods). We then recorded time-lapse, bright-field images of the plate such that pixel intensity is indicative of local cell density, with low intensities (darker regions) corresponding to high cell density. Though there are nonlinear effects, e.g., halos formed around large aggregates and issues of saturation, we focus on large structures formed in these movies which are largely insensitive to these issues.

We first observed that when the spotting cell density is very low ($5 \times 10^8$ cells/ml), cells exhibit no large-scale pattern formation. Over the first few hours, cells largely move independently, reversing frequently and with minimal cell-cell contact and interactions. After a sufficient amount of time, typically 6–8 hours, we observed the formation of spatially stable streams that exist mostly as monolayers of cells (as opposed to the many hundreds of layers that exist in the fruiting body) as reported in Thutupalli et al. [31]. At this low density, starving cells do not aggregate to form fruiting bodies regardless of

has typically used systems of self-propelled rods [32, 34]. In this paper, we modify instead a well-established minimal model [21, 22] of active Brownian particles (ABPs) by adding reversals (Fig. 2A). Each reversing ABP is modeled as a disk of radius $a$, with dynamics governed by overdamped Langevin equations of motion,

$$\dot{r}_i = v_0 \kappa_i(t) \hat{n}_i + \mu \sum_j F_{ij} , \quad \dot{\theta}_i = \sqrt{2D_r} \eta_i(t) , \quad (2)$$

where $r_i$ and $\hat{n}_i = (\cos \theta_i, \sin \theta_i)$ are the position and orientation of the $i$th disk. [2] describes the velocity of the $i$th particle as a function of its self-propulsion and steric interactions. The direction of self-propulsion is updated stochastically according to a random torque $\eta_i(t)$ of unit variance. In [2] we have modified the standard ABP model by incorporating reversals through a function $\kappa_i(t)$, which takes the values $\pm 1$, changing sign at times given by a Poisson process with a mean reversal frequency $f_{\text{rev}}$. The force $F_{ij}$ is purely repulsive and represents an excluded-volume interaction. We use a harmonic potential, with $F_{ij} = k(2a - r_{ij})\hat{r}_{ij}$ for $r < 2a$ and $F_{ij} = 0$ otherwise, where $r_{ij} = r_i - r_j / |r_i - r_j|$ (see Materials and Methods for parameters and other details of the simulations). Since we want to investigate the role of motility in aggregation, we do not describe motility in terms of molecular mechanisms, such as chemical signaling, and ignore other physical mechanisms such as attraction, alignment, and torque generation due to the elongated cell shape, and complex interactions with the substrate. Instead, particles only interact through repulsive forces and independently adjust their direction of self-propulsion.

Introducing a reversal frequency adds a new timescale to the ABPs, the effects of which can be included in an effective rotational diffusion via (see SI for a derivation)

$$D_{\text{eff}} = D_r + 2 f_{\text{rev}} . \quad (3)$$

Hence, particles travel with an orientational persistence time $\tau_r = 1 / D_{\text{eff}}$ and their random paths have a persistence length $l_p = v_0 \tau_r$. We can demonstrate this by measuring the mean squared displacement (MSD) for individual ABPs with different reversal frequencies (Fig. 2B). In agreement with our calculations, we find that the crossover between ballistic and diffusive motion occurs at precisely $\tau_r$ for all $f_{\text{rev}}$. Interestingly, this property holds for finite particle densities as well (Fig. 2C). Therefore, the effective diffusion coefficient, $D_{\text{eff}}$, is the appropriate parameter to use in [2] for calculating the inverse Péclet number for reversing ABPs.
the characteristic aggregate size \( L \), units of the snapshots shown in Fig. 1 are taken at equal times in is quantitative. This is demonstrated by the fact that \( M. xanthus \) or phase-separated. and simply refer to a configuration as either homogeneous ever, due to the difficulty of this type of analysis, we do experiments and simulations (Appendix and Fig. 7). How- ever, of aggregates via a nucleation and growth mechanism. This is shown in Figure 1C, for \( \phi = 0.29 \), where one can see that many small clusters soon start to form and dissolve (second snapshot, for \( t = 18\tau_1 \)). With time, some of these small clusters randomly develop into nucleation centers and grow, as can be seen in the snapshots for longer times.

When we further increased the initial culture density to \( 1 \times 10^{10} \) cells/ml and above, we observed that \( M. xanthus \) forms aggregates via a different dynamical mechanism (Fig. 1B). Rather than the spatially random nucleation and slow growth of individual fruiting bodies that occur stochastically in time, we observed that high density cultures spontaneously and immediately aggregate over the entire field of view. Within the first 6 hours after plating, we observed the formation of a global instability and small, mesh-like structures. As the mesh coarsens over time, small aggregates appear that are connected by less dense layers of cells. Finally, a subset of these aggregates grow in time and turn into round fruiting bodies. A similar aggregation process is observed in the ABP simulations (Fig. 1D). This kind of spontaneous phase separation is called spinodal decomposition and classically arises when microscopic fluctuations in the local density are inherently unstable with no energy barrier separating the mixed and more favorable phase-separated regimes.

A quantitative treatment of the distinction between the spinodal decomposition and nucleation and growth reveals that we do observe these two regimes in both experiments and simulations (Appendix and Fig. 7). However, due to the difficulty of this type of analysis, we do not attempt to distinguish these them in the following and simply refer to a configuration as either homogeneous or phase-separated.

It is important to stress that the comparison between \( M. xanthus \) aggregation and MIPS in our reversing ABPs is quantitative. This is demonstrated by the fact that the snapshots shown in Fig. 1 are taken at equal times in units of \( \tau_1 \). Additionally, we have quantified the growth of the characteristic aggregate size \( L(t) \) in Fig. 3. In reversing ABPs the length scale of coarsening in the spinodal regime grows as \( L(t) \sim t^{0.281 \pm 0.002} \), as found previously for non-reversing ABPs [38]. We measured the increase in length scale over time for a developing \( M. xanthus \) population (Methods) and found a power law with exponent \( 0.30 \pm 0.02 \), consistent with the value obtained from ABP simulation.

![Graph showing growth of the coarsening length scale with time for spinodal decomposition of both ABP simulations (red squares) and \( M. xanthus \) experiments (blue circles). Simula tion results are given in units of particle radius and the experiments are reported in microns, while times are written in units of \( \tau_1 \) for both cases (\( \tau_1 \approx 10 \) min for \( M. xanthus \)). Both coarse with a power law in time, with exponents \( \alpha_{\text{expt}} = 0.30 \pm 0.02 \) and \( \alpha_{\text{simul}} = 0.281 \pm 0.002 \).

The later stages of coarsening involve significant flux between neighboring fruiting bodies. This can be seen directly in some movies and through dynamics similar to the Ostwald ripening seen in passive systems, a surface-tension driven phenomena that causes smaller aggregates to dissolve into nearby larger aggregates. Recent work by Bahar et al. used a model of Ostwald ripening to describe the later stages of \( M. xanthus \) aggregation and accurately predicted the disappearance and persistence of aggregates [39].

### C. A phase diagram for aggregation

We have seen that ABPs and colonies of starved \( M. xanthus \) show similar aggregation patterns and that, in the phase-separated regime, aggregates grow with the same exponent. In order to demonstrate that \( M. xanthus \) aggregation is indeed an example of MIPS, we additionally need to show that the behavior is controlled entirely by density and by the effective inverse Péclet number:

\[
\text{Pe}^{-1}_\tau = \ell_c D_t^{\text{eff}} / v_0 = \ell_c (D_t + 2f_{\text{rev}}) / v_0. \tag{4}
\]

The density-Pe\(_{-1}\) phase diagram for ABPs has been obtained before by several authors [35, 38, 40]. Here we construct the phase diagram for reversing ABPs by studying the probability distribution of local particle density (Fig. 4A). In spatially homogeneous systems, this distribution is unimodal, with a peak at the total packing fraction \( \phi \). For phase-separated systems, the distribution is bimodal with peaks corresponding to the density of two coexisting phases (see Appendix for further information). The phase diagram for reversing ABPs coincides with that of standard ABPs when plotted in terms of the effective inverse Péclet number [4].

To control the Pe\(_{-1}\) of \( M. xanthus \) cells experimentally, we took advantage of the non-reversing mutant \( \Delta \text{FrzE}, \)
which does not change its velocity over time even in starvation conditions (Fig. 5). We then varied $Pe^{-1}$ by altering the propulsion speed $v_0$ using the drug nigericin [41]. We prepared samples at different densities and nigericin concentrations and imaged their aggregation dynamics, determining for each experiment whether phase separation had occurred (Methods and Appendix).

To calculate the inverse Pécel number for each experiment, we performed separate tracking experiments to probe $D_r$ and cell speed for each nigericin concentration. We mixed a small number of fluorescently-labeled cells with non-fluorescent cells at a ratio of 1:400 and tracked only the fluorescent cells to measure the speed. We estimated $D_r$ by tracking the motion of cells at very low density such that cells do not physically interact with each other. The decay of the velocity temporal autocorrelation function indicates that $D_r = 0.04 \pm 0.02 \text{ min}^{-1}$. We note that the typical reversal frequency of wild-type (WT) M. xanthus, $f_{rev} \sim 0.05 - 0.17 \text{ min}^{-1}$, is of the same order of magnitude but slightly larger than the rotational diffusion coefficient. This implies that subtle changes to $f_{rev}$ can have appreciable effects on $Pe^{-1}$. Had $D_r$ been much larger, cellular control of $f_{rev}$ would have been ineffective as a method to alter $Pe^{-1}$. We combine the measured speed and $D_r$ with the average cell size of $l_c = 2.5 \mu m$

1 While ∆FrzE cells have been reported to reverse at a very low frequency, we did not observe any reversals using this strain in our analysis.

D. Starving M. xanthus change velocity and reversal frequency to induce aggregation

The inverse Pécel number contains four parameters, two of which M. xanthus potentially has the ability to control during fruiting body formation. Cells do not grow during aggregation due to the starvation conditions and $D_r$ is presumably set by thermal fluctuations of the cell body and molecular noise in the motility process. However, both the cell speed $v_0$ and the reversal frequency $f_{rev}$ are under cellular control.

We tracked individual fluorescent WT cells in an aggregating population of density $1 \times 10^9$ cell/ml for the first 11 hours and found that when starved, WT cells change their gliding speed $v_0$ and reversal frequency $f_{rev}$. In the first 3-4 hours, WT cells exhibited low gliding speed $v_0 \approx 1.5 \mu m/min$. Additionally, under 10% of cells are actively moving during this period (Fig. 5), in agreement with previous reports of an initial “resting” phase [42]. After being starved for over 5 hours, WT M. xanthus cells speed up to $v_0 \approx 2.5 \mu m/min$ (Fig. 5A), almost doubling their initial $v_0$. At these times, over 90% of the cells are...
actively moving (Fig. 8). ∆FrzE cells, in contrast, do not change speed when starving, potentially indicating a link between the Frz molecular pathway and gliding speed. We also found that reversal frequency decreased significantly from 0.128 min⁻¹ to 0.055 min⁻¹ over the 11-hour experiment (Fig. 5B). A combination of increased $v_0$ and decreased $f_{rev}$ together give rise to a reduction of the inverse Péclet number from 0.60 to 0.16 (Fig. 5C).

This reduction in the inverse Péclet number is sufficient to drive aggregation and fruiting body formation. Before starvation, cells move slowly (or hardly at all) and reverse frequently, favoring a homogeneous population distributed throughout the surface (Fig. 4B black line). Upon starvation, wild-type cells speed up and reverse less often, producing a situation favorable for phase separation and fruiting body formation (Fig. 4B red line).

III. DISCUSSION

In this paper, we considered *M. xanthus* fruiting-body formation as a two-dimensional (2D) phase separation process and compared experimental results at different densities, speeds, and reversal frequencies to an ABP model with reversals. We find that *M. xanthus* aggregation is driven, at least initially, by a MIPS process, a generic feature of active matter systems. Importantly, this can happen in the absence of complex signaling mechanisms and interactions between cells, and requires very little real-time control at the cellular level. While the ability to actively change motility ultimately leads to a phase transition, cells do not have to implement a complicated feedback mechanism to alter motility in response to specific chemical or mechanical cues. Rather, cells need only speed up and suppress reversals upon starvation and the collective mechanics then naturally induces phase separation of the entire population.

We note that our simple ABP model is meant to capture the most basic underlying driving forces of aggregation. Many of the details that we purposely left out of our simulations most likely do play a role in the specific evolution and shape of the final fruiting bodies. These include cell-cell alignment, the effects of “slime following,” and cell-cell communication via C- and A-signaling mechanisms. More complicated models of *M. xanthus* aggregation may uncover the role of these additional parameters [43], however, we feel it is unlikely that they will change the basic features we have observed here.

Finally, our ABP simulations consider a 2D system of particles while developing *M. xanthus* cells form three-dimensional (3D) aggregates. In simulations, aggregates form because particles impede each other’s motility and jam together. While this has also been observed in 3D simulations of swimming ABPs [44], the 3D aggregation process in *M. xanthus* is a dewetting phase transition where the population goes from a fully wetted phase to a dewetted phase. The population forms droplets on the surface, similar to water on a hydrophobic surface.

While we don’t expect this difference to change the very basic mechanism for phase transition we propose here, the details could prove important. Future work tracking cells and monitoring aggregate shape in three dimensions should lead to a more accurate, 3D theory of fruiting body formation.

IV. METHODS

A. *Myxococcus xanthus* culture and development conditions

Liquid cultures of wild-type *M. xanthus* strain DK1622 and ∆FrzE were grown at 32°C in agitating CTTYE medium (1.0% Casitone, 0.5% yeast extract, 10.0 mM Tris-HCl at pH 8.0, 1.0 mM KH₂PO₄, and 8.0 mM MgSO₄). Kanamycin (40 μg/ml) was added only to liquid cultures of ∆FrzE. Starvation assays were performed using non-nutritive Tris phosphate medium (TPM) agarose (10.0 mM Tris-HCl at pH 7.6, 1.0 mM KH₂PO₄, 8.0 mM MgSO₄, and 1.5% agarose). To induce development, growing cells were harvested from liquid culture at mid-log phase and resuspended to a final concentration of various densities in TPM: 5 × 10⁷, 1.5 × 10⁸, 2.5 × 10⁸, 5 × 10⁸, 2.5 × 10⁹ cells/ml. 10 μl spots were plated on a TPM agarose slide complex and allowed to dry as described previously [39]. To modulate velocity, cells suspensions and TPM agarose was treated with nigericin sodium salt at concentrations of 0, 1, 2, 4, and 10 μM.

B. Imaging and tracking

Cells were imaged at 20× and 100× magnification to record the behavior of both single cells and aggregates. For 100× magnification, cells were imaged on a modified Nikon TE2000 inverted microscope with an oil-immersion objective (NA 1.49). To capture an enlarged 110×110μm² field of view, we used a tiling strategy and imaged a 3×3 grid of 100× fields. Details of this imaging and auto-focusing strategy were reported previously [31]. Images were recorded at a frame rate of 10 seconds. Cell tracking using bright field images was performed using our previously published BCTracker algorithm [31].

For high cell density fluorescence cell tracking, a 1:400 mixture of Alexa Fluor 594 carboxylic acid succinimidyl ester labeled DK1622 or ∆FrzE cells to non-labeled cells was used to record the behavior of individual cells in large groups. To stain cells, cells were grown to mid-log, harvested by centrifugation and resuspended in MC7 buffer. 2 μl of dye (10 mg/ml, dissolved in DMSO) and 5μl of 1M NaHCO₃ was added to 100 μl of cells and shaken vigorously at 100 RPM for 1 hour in the dark at room temperature. Cells were then pelleted by centrifugation, washed 3 times in TPM and microscopically examined. Fluorescent microscopy images were taken at a rate of one
frame per min for the first 15 min in each hour to minimize the amount of laser exposure for cells. Experiments lasted 11 hours. Fluorescent cells were tracked using a particle tracking algorithm developed by Crocker, Grier and Weeks [45]. In our analysis, a cell is counted as actively moving if during each tracked hour it glides with a mean speed greater than 0.5 \( \mu \text{m/min} \). A reversal event is defined as occurring when the velocity vector between two successive time points changes sign.

To calculate the rotational diffusion constant, we tracked 50 \( \Delta \text{FrzE} \) cells and observed their motion for at least 35 \( \mu \text{m} \). The diffusion constant was then calculated from the decay of the velocity temporal autocorrelation function and assumed to be the same for all experiments (Fig. 8). To measure the length-scale growth displayed in Fig. 3, we imaged WT \( M. \text{xanthus} \left( 1 \times 10^{11} \text{cells/ml} \right) \) on a 20 \( \times \) magnification home-built bright field microscope at frame rate every 10 second for 24 hours.

At higher densities, it is experimentally difficult to break up cell clumps that have formed in the liquid culture. Thus, at the beginning of a movie we sometimes see isolated aggregates that are not fruiting bodies and which very quickly dissolve as cells migrate out of them. We start our analysis from the point where the initial visible aggregates have dissolved.

C. Simulation details and parameters

We simulated (2) using a standard Brownian Dynamics algorithm in an \( L \times L \) box with periodic boundary conditions. In all cases, we use \( k = \mu = 1 \) so the interaction timescale \( \tau_D = (\mu k)^{-1} \) sets the unit time and we use the particle radius \( a \) as the unit of length \( (a = 1) \). To prevent particles from passing through each other, we set \( v_0 = (a \mu k)/100 \). We fix the packing fraction \( \phi = N \pi a^2 / L^2 \), which sets the total number of particles, \( N \). The rotational diffusion \( D_r \) and the reversal frequency \( f_{\text{rev}} \) are varied to obtain the desired \( \text{Pe}^{-1} \).

For each set of parameters, we average over 10–100 runs and use a jackknife method [38, 46] to estimate statistical errors. In order to compute the length scale \( L(t) \) and its coarsening exponent (Fig. 3) we used a large system size with \( L = 1000 \), \( \text{Pe}^{-1} = 0.01 \) and a packing fraction of \( \phi = 0.5 \) \( (N = 159, 154 \text{ particles}) \), averaging over 100 independent runs. The same box size was used to generate the movies and snapshots of Fig. 1. For other quantities we did not need such a large system size. The phase diagram was computed on systems with \( L = 200 \) (10 runs for each set of parameters).

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V. APPENDIX

A. Effective rotational diffusion in the presence of directional reversals

We consider the overdamped dynamics of a single self-propelled particle with directional reversals in two dimensions. The directional unit vector $\mathbf{n} = (\cos \theta, \sin \theta)$ is set by the angular direction $\theta \in [0, 2\pi]$. This evolves in time as

$$d\theta = \sqrt{2D_\theta} dW_t,$$

where $D_\theta$ is rotational diffusion and $W_t$ is a Wiener process. Particle direction periodically reverses as a Poisson process where the waiting time distribution between two reversals is exponential. The overdamped equation of motion is expressed as a stochastic process driven by dichotomous Markov noise (DMN) [47],

$$d\mathbf{x}(t) = v_0 \mathbf{n}(t) d\xi_t,$$

where $\xi_t \in \{-1, 1\}$ is the symmetric DMN with zero mean and exponential correlation $E[\xi_t, \xi_{t'}] = \exp(-2f_{rev}|t - t'|)$, and $f_{rev}$ is the mean reversal rate. Particle position $(x(t), y(t))$ evolves as

$$x(t) = x_0 + v_0 \int_0^t d\xi_t' \cos \theta(t')$$
$$y(t) = y_0 + v_0 \int_0^t d\xi_t' \sin \theta(t'),$$

where $(x_0, y_0)$ is the initial position. Since $\theta(t)$ and $\xi_t$ are independent random variables, we separately average over all realizations of the rotational noise and initial angular conditions and then average over all realizations of the DMN to calculate the mean-square displacement

$$\langle |x(t) - x(t')|^2 \rangle = \frac{v_0^2}{2D_\theta} \left[ t - \frac{1}{D_\theta} (1 - e^{-D_\theta t}) \right],$$

where $D_\theta^\text{eff} = D_\theta + 2f_{rev}$ is the effective rotational diffusion.

B. Measuring $L(t)$

Experiment: In experiments, we have obtained a digital image of the system for each time step measured. Local density is determined by the intensity of light at each pixel, where density has an inverse relationship with light intensity (darker is more dense). Once the experimental $S(k)$ distribution has been obtained, it is fit with an exponential decay added to a Gaussian function as shown in Figure 6 (left), and the mean wave number is taken to be the inverse of the dominant length scale of the system at the time of the snapshot.

Simulation: In simulations, we discretize the system by dividing it into bins with width equal to the particle radius $a$ and assigning a binary 1 or 0 value to each bin depending on whether or not a particle is centered in it. In order to reduce noise, the resulting density distribution is averaged over exponentially increasing temporal bins [38]. The FFT is then computed in 2D to produce the structure factor $S(k, t)$ (Fig. 3, right). The average length scale was calculated from the first moment of $S(k, t)$

$$L(t) = \frac{\int_{k_{\text{max}}} S(k, t) \, dk}{\int_{k_{\text{max}}} k S(k, t) \, dk},$$

where $k_{\text{max}}$ is chosen to exclude noisy, high-frequency modes and sample the mean of the lowest-wavenumber peak in $S(k)$. We do this for each range of times following a Jackknife algorithm over bins that are logarithmically decreasing as we go backwards in time.

C. Distinguishing phase behavior

To quantitatively distinguish between nucleation and growth phenomena and spinodal decomposition, we take advantage of the temporarily spontaneous nature of the spinodal decomposition phase transition. In experiments, we monitor formation and growth of aggregates. We measured the distribution of times at which aggregates reach one half of their final size. The distribution of variances from these experiments yields a clear lower peak at $V_{\text{avg}}(t) < 1h$ (Fig. 4, A-B), corresponding to movies in which spinodal decomposition occurs. The higher-value peaks correspond to nucleation and growth where aggregates form randomly over time.

We performed a similar calculation using the simulations. Because we are limited by the simulation size, in many cases we only get one cluster at the end. To probe if a system undergoes a spontaneous transition for each set of parameters, we performed 100 simulations and compared the cluster growth across simulations (Fig. 7). For sets of parameters that lead to spinodal decomposition, the distribution of aggregation times has a clear peak, while for nucleation and growth a large tail develops (with an eventually diverging average as we approach the phase boundary).

D. Producing the phase diagram with simulations

Here, we follow a common technique for mapping the phase space of ABPs [24], which utilizes the distribution of local densities in the final state of a simulation runs. In simulations, we utilized the distribution of local densities in the final state of a simulation to locate the phase boundaries. In this technique, a homogeneous state yields a unimodal density distribution while phase-separated states produce a bimodal density distribution (Fig. 9). We sampled the local density in square windows of width $L_W = 20a$ (the total size of the system...
is $L = 200a$). For close-packed particles, a window contains $\approx 115$ particles, resulting in a density distribution histogram with bin width $\approx 0.01$.

We sample the last half of each run by first filling local histograms then averaging over 10 runs select coexisting local densities by the peaks of the distribution. With enough sampling, we find only two possible distributions: unimodal (homogeneous) and bimodal (phase separated), and we plot the resulting peaks in our phase diagram to show the coexistence peaks.
FIG. 6. Left: 2D FFT of WT *M. xanthus* movie averaged over 30 lines and fitted to an exponential decay and a Gaussian at time $t = 1, 2, 3, 5, \text{ and } 10 \text{h}$. Right: Static structure factor calculated for time intervals used to produce $L(t)$ ($\phi = 0.5, Pe^{-1} = 0.01, L = 10^3$). This is an example of spinodal phase decomposition, where this distribution is expected to tighten around smaller $k$ as dense regions coarsen with time.
FIG. 7. (A) Examples of WT fruiting body size evolution at different densities. The top two conditions stimulate nucleation and growth as fruiting bodies form at varying times. The bottom two conditions stimulate spinodal decomposition where the emergence of fruiting bodies is synchronized. (B) Histogram of the variance in the time when fruit bodies reach half-maximal size. When this time variance is smaller than 1h, we conclude that the system is undergoing spinodal decomposition. (C) The fraction of clusters observed over time is shown for 100 simulations at varying packing fractions \( P_{\text{r}^{-1}} = 0.01 \). The resulting deviation of the nucleation times shows how as density is decreasing close to the inside of the coexistence line, significantly longer mean times and wider variances in times of nucleation occur, resulting in observable differences between spinodal decomposition and nucleation and growth.
FIG. 8. (A) Top: Examples of ∆FrzE cell tracking results. Bottom: Velocity temporal autocorrelation function. An exponential fit to the data yields a rotational diffusion coefficient \( D_r = 0.04 \pm 0.02 \text{ min}^{-1} \). (B) Tracking of ∆FrzE cells under various Nigericin concentrations. Cell velocity is averaged over \( \approx 50 \) tracks each. (C) Active cell percentage of fluorescent tracking of both WT and ∆FrzE cells. Note that ∆FrzE cells are active even in the early starving stage, while WT cells undergo a “resting” phase in the first 5 hours.

FIG. 9. Local density distributions for three different mean densities, \( \phi_l = \{0.45, 0.55, 0.65\} \), at three different rotational Peclet numbers, \( Pe^{-1}_r = \{0.005, 0.0025, 0.00125\} \). The peaks of these distributions are used to draw the boundary in the simulation phase diagram.