Imaging the emergence of bacterial turbulence using light-powered *Escherichia coli*

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Using genetically engineered light-powered *E. coli* whose locomotion can be reversibly controlled by light, we study the emergence of collective bacterial swimming, a phenomenon often refereed to as bacterial turbulence. Bacterial turbulence is triggered in our experiments by adjusting light intensity, allowing for real-time imaging of the onset of collective motions in concentrated bacterial suspensions. We systematically map the phase diagram of the 3D flow of bacterial suspensions over a large control parameter space spanned by bacterial concentration, the swimming speed of bacteria and the number fraction of active swimmers and delineate the phase boundary between the disordered phase and the turbulent phase. The diagram quantitatively agrees with a simple model balancing hydrodynamic interactions with rotational diffusion of bacteria. We further identify two different kinetic pathways towards bacterial turbulence. Near the phase boundary, one-step transitions with long incubation periods are observed, whereas two-step transitions driven by a long-wavelength instability are found deep inside the turbulent phase. The microscopic origin of these unusual kinetics is finally discussed. Our experiments verify the key predictions of existing theories on transition points and the mode of instability and reveal unexpected kinetic features of nonequilibrium phase transitions in active matter.

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I. INTRODUCTION

Collective motions of biological systems such as bird flocks, fish schools and bacterial swarms are the most vivid examples of the emergent behaviors of active matter. While moving independently at low density, self-propelled units in active matter can move collectively at high density, giving rise to coherent flows at length scales much larger than the size of individual units. In bacterial suspensions, these coherent flows manifest a chaotic pattern of intermittent vortices and jets, reminiscent of turbulent flows at high Reynolds numbers. Hence, the flows induced by bacterial collective swimming are often called as active or bacterial turbulence.

Extensive theoretical and numerical studies have been conducted in understanding the physical principles underlying the nonequilibrium transition between the disordered and the turbulent states in bacterial suspensions. Particularly, mean-field kinetic theories have been developed by extending the classic models of suspensions of passive rod-shaped particles. Subramanian and Koch showed that the isotropic disordered state of suspensions of pusher swimmers like *E. coli* becomes unstable above a threshold concentration \( n_c \) that is inversely proportional to the activity of swimmers as quantified by their swimming speed \( v \). Independently, Saintillan, Hohenegger and Shelley showed that a long wavelength hydrodynamic instability drives the transition to active turbulence in pusher suspensions. The transition kinetics was further studied beyond the linear regime by numerically solving kinetic equations and simulations.

In comparison, definitive experiments on bacterial suspensions that can quantitatively verify theoretical predictions are still few and far between. Pioneering experiments on suspensions of *Bacillus subtilis* first showed the emergence of bacterial turbulence at high concentrations in absence of bioconvection and chemotaxis. Various physical properties of bacterial turbulence such as density fluctuations and coarsen and defects structures and mass and momentum transports have been subsequently studied. While most of these studies focused on the rise of bacterial turbulence with increasing bacterial concentrations, few experiments have considered the effect of other important factors such as the swimming speed of bacteria and the presence of defective immobile bacteria on the bacterial turbulent transition. A detailed mapping of the phase diagram of 3D bacterial flows over a large control parameter space is still lacking. Such a phase diagram is crucial to quantitatively verify the theoretical predictions on the transition point including \( n_c \sim 1/v \) from the kinetic theories. Particularly, the study of the effect of doping immobile cells on the phase dynamics will provide important evidence to resolve the long-standing controversy over whether hydrodynamic or steric interactions play the dominant role in inducing the collective motion of swimming microorganisms. While the presence of immobile bacteria would reduce the mean active hydrodynamic stress in bacterial suspensions and thus suppress the hydrodynamics-induced collective motion, the steric interaction arising from the rigid rod-shaped body of immobile cells would promote the collective motion at a given concentration of active particles as demonstrated by experiments on dry active granular matter.
In addition to the phase dynamics, the kinetic pathway towards the collective motion of swimming microorganisms also remains largely unexplored. Kinetics of a phase transition affect both the transition rate and the structure of intermediate states and is of great importance in understanding the phase dynamics of equilibrium systems [42, 43]. It plays an equally important role in a nonequilibrium phase transition, which can provide the crucial information on the rise of the nonequilibrium order from interparticle interactions and reveal the nature of the transition. Hence, resolving the route to bacterial turbulence—a premier example of collective motions—addresses the central question in active matter: how do random self-propelled units self-organize into large-scale dynamic structures?

II. RESULTS AND DISCUSSION

In this study, we fill the important knowledge gap and experimentally investigate both the phase dynamics and kinetic route of bacterial turbulence. Our study provides not only the most comprehensive phase diagram of the flow of 3D bulk bacterial suspensions hitherto, but also the detailed quantitative characterization of the kinetics of the bacterial turbulent transition. In order to explore a large control parameter space, we use three different strains of *E. coli* in our experiments, which all share similar body plans (Materials and methods). In addition to a wild-type strain (BW25113), a strain of tumblers with *cheZ* knocked out (RP1616) is used as immobile passive particles. Moreover, a strain of light-powered *E. coli* (BW25113 with proteorhodopsin, a light-driven transmembrane proton pump [45, 46]) is also used, whose mobility varies with green light of different intensities (Fig. S1 in [44]). By mixing different strains of bacteria and controlling light intensity, we explore the phase diagram of 3D bacterial flows spanned by the total bacterial concentration, the average swimming speed of bacteria and the number fraction of active swimmers (Materials and methods).

A. Transition to bacterial turbulence

The collective motion of bacteria leads to bacterial turbulence with characteristic intermittent jets and whirls, whose lengths and speeds are much larger than those of individual bacteria (Figs. 1a, b and SI Video 1). To quantify bacterial turbulence, we calculate the orientational correlation of local velocities, 

$$c_i = \text{min}_{j=1,4}(\hat{v}_i \cdot \hat{v}_j),$$

where \(\hat{v}_i\) is the unit vector along the direction of the local velocity at position \(i\) and \(\hat{v}_j\) is the direction of the velocity in one of its four neighboring boxes adopted in Particle Imaging Velocimetry (PIV) (Materials and methods). We identify a local region with high velocity orientational correlation when \(c \geq 0.9\). The area fraction of these highly correlated regions, \(\phi\), is used in our study as an order parameter quantifying the rise of bacterial turbulence (Fig. 1c). This choice is similar to that used in previous studies [28]. Quantitatively similar results can also be obtained if the average orientational correlation \(\langle c \rangle\) or the velocity correlation length is used as the order parameter (Fig. S2 in [44]). In addition to the velocity orientational order, we also measure the strength of bacterial flows via energy density, 

$$E = (\bar{v}_x^2 + \bar{v}_y^2)/2,$$

where \(\bar{v} = (v_x, v_y)\) is the in-plane velocity of 3D bacterial flows.

A transition to bacterial turbulence is observed as we increase bacterial concentration, \(n\) (Fig. 2a). A sharp increase of \(\phi\) occurs around \(n = 10n_0\), where \(n_0 = 8 \times 10^8\) ml\(^{-1}\) is the *E. coli* concentration at OD\(_{600}\) of 1.0 close to the natural bacterial concentration in the intestinal tract [47]. The transition point, \(n_c\), is then obtained from the inflection point of the error function fitting of
FIG. 2: Transition to bacterial turbulence and the phase diagram of 3D bacterial flows. (a) Velocity orientational order $\phi$ and flow energy $E$ as a function of bacterial concentration $n$ of wild-type bacteria with bacterial swimming speed $v = 28.7 \mu m/s$ and swimmer fraction $f = 1$. Black squares are for $\phi$ and red circles are for $E$. (b) $\phi$ and $E$ as versus $f$ at fixed $n = 30n_0$ and $v = 28.7 \mu m/s$. (c) $\phi$ and $E$ as versus $v$ at $n = 60n_0$ and $f = 1$. Black lines indicate error function fittings. (d) The phase diagram of bacterial flows in the phase space of $n$, $v$ and $f$. Red circles indicate the turbulent phase, whereas black squares indicate the disordered phase. The surface is the model prediction (Eq. 1). (e) The rescaled phase boundary between the disordered and the turbulent phases at different $v$ (indicated in the figure). The black line is the model prediction (Eq. 1).

$\phi(n)$. Coincidently, the flow energy $E$ also increases sharply around $n_c$. We find similar sharp transitions to bacterial turbulence when increasing $v$ and $f$ at fixed $n$ (Figs. 2b, c). Particularly, bacterial turbulence is observed at $f = 20\%$ for the mixture of wild-type *E. coli* ($v = 28.7 \mu m/s$) and immobile bacteria at $n = 100n_0$. Such a low $f$ demonstrates the robustness of the collective flow of bacterial suspensions, greatly surpassing the state-of-the-art design of active robotic systems [48]. The high tolerance to malfunctioning units likely arises from long-range hydrodynamic interactions, lacking in dry active matter [3].

B. 3D phase diagram

Systematic measurements over a thousand bacterial samples under different combinations of control parameters yield a full 3D phase diagram of bacterial flows, where bacterial turbulence emerges at large $n$, $v$ and $f$ (Fig. 2d). It should be emphasized that, although the transition to bacterial turbulence has been investigated with increasing $n$ and $v$ in different experiments [26, 28, 30, 31], to the best of our knowledge, systematic measurements over such a large parameter space in the same experimental system have not been achieved previously. This comprehensive 3D phase diagram not only allows us to quantitatively verify theoretical predictions that has not been possible before, but also sets up an indispensable framework for exploring the kinetics of the transition in the next section.

We estimate the phase boundary between disordered and turbulent phases based on a simple hydrodynamic model, where two-body hydrodynamic interactions that promote local bacterial alignment compete with random rotations that disorientate bacteria [49]. To consider the presence of immobile bacteria, we have also included the higher-order hydrodynamic interactions from reflection flows in the model [44]. Transition concentration $n_c$ is then predicted as

$$n_c - n_0 = \left( \frac{n_c}{n_0} \right)^2 \frac{1}{f v},$$

where $r_0 = 1.6 \mu m$ and $v_0 = 296 \mu m/s$ are geometric and dynamic factors related to the shape and activity of bacteria [44]. Eq. 1 allows for the collapse of the transition boundaries under different control parameters and shows a quantitative agreement with experiments (Fig. 2e).

We compare our experiments with kinetic theories. First, since $n_0 r_0^3 \sim 0.003$, the square term on the left...
of Eq. 1 is negligible when $n_c/n_0$ is not too large, which leads to $n_c \sim 1/v$, directly confirming the prediction of kinetic theories 12, 18. Second, at $f = 1$, we find $n_c$ ranges between 12$n_0$ and 40$n_0$ for different bacterial velocities. The average distance between bacteria at $n_c$ is about 4 to 6 $\mu$m, comparable to the size of a single bacterium with its extended flagellar bundle 47. Short-range hydrodynamic and steric interactions are supposed to be important at such small scales 16. Hence, it is rather surprising that the far-field dipolar interaction adapted in our simple calculation is sufficient to describe the experimental phase boundary quantitatively, which indeed supports the basic assumption of kinetic theories 12, 13, 18. Consistent with this finding, increasing the fraction of immobile bacteria at either a fixed total concentration $n$ or a fixed concentration of active swimmers $n_f$ suppresses bacterial turbulence in our experiments, rather than promoting the collective motion as observed in dry active matter with purely short-range steric interactions 41. Lastly, it is worth noting that the size of samples in our experiments is fixed with the minimal dimension between 150 $\mu$m and 170 $\mu$m, much larger than the size of single bacteria. The volume of the samples is on the order of several microliters, which contains more than $10^7$ bacteria at $n = 10n_0$. Simulations and kinetic theories have shown that $n_c$ is independent of the system size at such large scales 18. Our previous experiments have shown that the correlation length of collective flows saturates for large systems above 140 $\mu$m 32. More recent experiments have also shown that the onset of collective motions and bacterial superfluids is insensitive to the system size above 170 $\mu$m 50. Thus, the phase diagram shown in Fig. 2d should approximate the phase behavior of bacterial suspensions in the bulk limit.

C. Transition kinetics

Next, using the phase diagram as a roadmap, we explore the kinetics of bacterial turbulent transition, which reveals the mode of instability and the transient structure of bacterial suspensions through the transition. To study the kinetics, we trigger the onset of bacterial turbulence by suddenly ramping up light intensity at $t = 0$, which increases bacterial swimming speed from that below the phase boundary to high $v$ above the boundary at given $n$ and $f$ (SI Video 2). Although individual bacteria in dilute suspensions recover their swimming speeds within a couple of seconds (Fig. S1 in 14), the emergence of collective flows can take much longer times depending on the specific control parameters. In the region above but close to the phase boundary, the transition exhibits a surprisingly long incubation period with low velocity orientational order $\phi$ and flow strength $E$ ($\sim 100$ s in Fig. 3a). During incubation, localized regions with relatively large velocity orientational correlation (high $c$) nucleate within the disordered phase (Fig. 3b). The incubation period witnesses the slow growth and the fluctuation of these high-$c$ regions. After incubation, the high-$c$ regions grow quickly and eventually percolate the entire system.

The incubation period becomes increasingly short as we move away from the phase boundary. Far above the
boundary, the increase of the velocity orientational correlation is almost instantaneous across the entire system after the light ramp (Figs. 3b–d), whereas the energy of the flow remains low at beginning and grows to a steady-state plateau at a much later time. Thus, the system exhibits a transient state with high velocity orientational order but low flow energy (the middle two pictures at \( t = 10 \) and 30 s in Fig. 3b).

A smooth crossover from the single-step transition to the two-step transition with the transient intermediate state can be seen from the \( E - \phi \) plot (Fig. 4a): \( E \) increases linearly with \( \phi \) near the phase boundary at low \( n \) indicating the concurrent increase of the two quantities, whereas L-shaped \( E - \phi \) relations are observed for the two-step transition deep inside the phase boundary at high \( n \). The fast increase of \( \phi \) at early times results in the flat region of the curves at low \( E \).

We further quantify the time and length scales associated with transition kinetics. The transition rate is measured by the incubation time, \( \tau_{in} \), defined in analogy of nucleation and growth processes in equilibrium phase transitions (Fig. S3 in 4a). \( \tau_{in} \) decreases with \( n \) and reaches a low plateau of \( \sim 10 \) s above \( 60n_0 \) (Fig. 4b), comparable to the time it takes for a single bacterium recovering its swimming speed in the dilute limit. The presence of the transient state with high velocity orientational correlation and low flow energy is characterized by the time difference, \( \Delta \tau = \tau_{E} - \tau_{\phi} \), where \( \tau_{E} \) and \( \tau_{\phi} \) are the times when \( E \) and \( \phi \) reach their steady states, respectively. \( \Delta \tau \) increases with \( n \) and can be as long as 65 s above \( 90n_0 \) deep inside the phase boundary. Note that although we discuss transition kinetics with increasing \( n \), qualitatively similar trends are also observed when \( v \) and \( f \) are varied (Figs. 3b–f).

The length scales associated with the transition kinetics are revealed by the energy spectrum of bacterial flows, \( E(k) \), where \( k \) is the wavenumber (Materials and methods). \( E(k) \) is related to the energy density \( E \) through \( E = \int_0^\infty E(k)dk \). Near the phase boundary, the increase of \( E(k) \) initiates at large \( k \) (or short wavelengths) and then propagates to small \( k \) over time (Fig. 4c), consistent with the scenario of nucleation and growth. In contrast, the spectrum increases significantly faster at small \( k \) deep inside the phase boundary at early times (Fig. 4d), indicating a long-wavelength instability. Such a long-wavelength instability qualitatively explains the two-step transition. First, the instability at small \( k \) naturally leads to a long-range velocity orientational correlation and, therefore, the fast increase of \( \phi \) at early times. Second, since \( E \) is determined by the integral of \( E(k) \) over all \( k \), the early-time increase of \( E(k) \) at small \( k \) does not substantially affect the flow energy, which shows strong increase only when \( E(k) \) at high \( k \) starts to grow at later times. As such, our kinetic measurements directly confirm the key prediction of kinetic theories on the existence of a long wavelength instability in suspensions of pusher swimmers [19–22].

D. Microscopic view

Lastly, we examine the evolution of the long-wavelength instability from the perspective of the dynamics of single bacteria using high-resolution fast confocal microscopy (Materials and methods). We find that the two-step transition deep inside the phase boundary can be understood microscopically from the development of the orientational order of bacteria in emerging turbulent flows. Directly tracking the orientation of individual bacteria in dense 3D suspensions is subject to large experimental errors. In order to quantify the local nematic
order of bacteria without tracking individual bacteria, we perform Fast Fourier Transform (FFT) on local regions (20 × 20 μm²) of confocal images. The degree of the local nematic order can be characterized by the anisotropy of the resulting FFT patterns, $F$ (Fig. 6 inset) (Materials and methods). Our measurements show that the local bacterial nematic order is linearly proportional to the flow energy $E$, but decouples from the velocity orientational order $\phi$ in the two-step transition (Figs. 6c, d).

The finding suggests the following physical picture. At early times, a slight deviation from the isotropic distribution of bacterial orientations induced by the long wavelength instability is sufficient to establish a strong velocity orientational correlation, where bacteria appear to orientate randomly in the emergent turbulent flow as shown in Fig. 6a. This picture further corroborates the exceptional robustness of bacterial turbulence identified from the phase diagram. Over time, the flow energy gradually increases as bacteria align more and more with the local flows and their neighbors (Fig. 6b). This observation directly verifies the numerical solution of the kinetic equations in the nonlinear regime [20], where the alignment of pusher swimmers with the direction of local flows develops progressively through the transition and the increasing alignment then further enhances the velocity of local flows. A strong steady-state turbulence is finally established when bacteria well align with their neighbors as shown in Fig. 6b), which gives rise to a large flow energy and a high local bacterial nematic order. Thus, our confocal measurements provide a microscopic view of the two-step kinetics induced by a long wavelength instability: the disordered phase (low $\phi$, low $E$, low $F$) → the disordered turbulent phase (high $\phi$, low $E$, low $F$) → the ordered turbulent phase (high $\phi$, high $E$, high $F$).

### III. CONCLUSIONS

We experimentally studied the emergence of the collective motion of bacterial suspensions, the so-called bacterial turbulence. By using three different $E. coli$ strains and examining over a thousand of bacterial suspensions at different concentrations, swimming speeds and fractions of active swimmers, we systematically mapped the phase diagram of 3D bulk bacterial flows over a large parameter space in one single experimental system. Based on a simple model balancing the hydrodynamic interactions and the random diffusion of bacteria, we presented a quantitative description of the phase boundary between the disordered and the turbulent phases and drew a detailed comparison with theoretical predictions that has not been possible before. Our experiments supported the basic assumption of the kinetic theories that the formation of the collection motion of swimming microorganisms is dominantly driven by the hydrodynamic interactions between bacteria, where the presence of immobile cells suppress, instead of promoting, the collective swimming in bulk bacterial suspensions.

Using the light-powered $E. coli$, we triggered the onset of bacterial turbulence and imaged the kinetics of bacterial turbulent transition in real time. Two distinct kinetic pathways were discovered, which exhibit different transition rates and structures of the transient state. Near the phase boundary, the transition shows a feature of nucleation and growth with a long incubation period. Deep inside the phase boundary, the transition takes two steps with a transient state of high velocity orientational order but low flow energy. Moreover, we illustrated the existence of a long wavelength instability in pusher suspensions deep inside the phase boundary and thus confirmed the key prediction of the kinetic theories. Our microscopic measurements further revealed the development of the instability in terms of the dynamics of single bacteria and showed that the transient state in the two-step transition is characterized by a low bacterial nematic order. These results demonstrated the remarkable robustness of bacterial turbulent flows and verified the kinetic theories from both macroscopic and microscopic perspectives. Taken together, our study provided a solid experimental benchmark on the collective motion of swimming microorganisms and addressed fundamental issues on the kinetics of nonequilibrium phase transitions in active matter.
FIG. 6: Confocal microscopy of the turbulent transition deep inside the turbulent phase at $t = 10$ s (a) and 30 s (b) after the light ramp. The incubation time of the transition $\tau \approx 6$ s. Bacterial concentration $n = 80n_0$, speed $v = 8.2 \mu m/s$ and swimmer fraction $f = 1$. Individual bacteria identified manually are marked with ellipsoids, whose orientational angle is indicated by the color shown in the inset of (a). The inset of (b) shows the FFT of the local region indicated by the dashed box. The aspect ratio of FFT patterns averaged over all the local regions, $F \equiv a/b$, is used to quantify the local nematic order of bacteria (Materials and methods). The dashed box is $20 \times 20 \mu m^2$. (c) Velocity orientational order, $\phi$, flow energy, $E$, and $F$ through the transition shown in (a) and (b). (d) $E$ versus $F$ at different control parameters (indicated in the figure). The open squares are for a system close to the phase boundary with a one-step transition, whereas the blue triangles are for a system deep inside the phase boundary with a two-step transition. The red disks are for a crossover system between the two limits. Different from the $E - \phi$ plots shown in Fig. 4, all the data collapse into a master curve showing the linear relation between $E$ and $F$.

Finally, our study also opens important questions for future experimental and theoretical development. First, it is still unclear on the microscopic origin of the long incubation period in the one-step transition near the phase boundary. Our preliminary study indicates that the incubation process may be influenced by the formation of bacterial clusters in suspensions. Nevertheless, the incubation time can be significantly longer than the time scale associated with the dissolving of bacterial clusters close to the phase boundary, suggesting the generic nature of the phenomenon. To understand the nucleation and growth process with long incubation requires prolonged experiments beyond the typical time of our experiments due to the problem of photobleaching and poses an experimental challenge for future studies. On the other hand, how to incorporate such an incubation process in kinetic theories presents perhaps an even more challenging theoretical question. Second, the long wavelength instability observed in our experiments indicates a system-size dependence of phase dynamics in confined systems. The change of the phase diagram and the kinetic pathway with the system size needs to be further confirmed and quantitatively measured in future experiments. Lastly, it is certainly worth of investigating the kinetic route to collective motions in other active fluids such as active cytoskeletons and suspensions of colloidal swimmers and examining whether the kinetic features observed in our experiments are generic for active matter or unique for microbiological systems.

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APPENDIX A: MATERIALS AND METHODS

1. Bacteria

Three strains of $E. coli$ are used. (i) A wild-type $E. coli$ K-12 strain (BW25113), which shows normal run-
and-tumble motions with an average swimming speed \( v = 28.7 \, \mu m/s \). (ii) A “tumbler” strain, which is made by knocking out cheZ of a wild-type E. coli strain (RP1616). The \( \Delta cheZ \) mutant shows constant tumbling motions. We use tumblers as passive particles. To deactivate the bacteria, we remove the flagella of tumblers by pipetting tumblers suspensions through a narrow pipette tip a few tens of times. (iii) A light-powered E. coli, whose swimming speed can be reversibly controlled by light intensity. We introduce a light-driven transmembrane proton pump, proteorhodopsin (PR), to wild-type E. coli (BW25113) by transforming the bacteria with plasmid pZE-PR encoding the SAR86 \( \gamma \)-proteobacterial PR-variant [44]. The activity of PR is directly correlated with the light intensity. Thus, we can control the swimming speed of bacteria using light of different intensities. The quantitative relation between the light intensity (in terms of the power of the light source) and the average swimming speed of bacteria is measured by tracking the motion of bacteria in dilute suspensions (Fig. S1a in [44]). The response time of the bacteria is fast within a couple of seconds after the change of the light (Fig. S1b in [44]). Note that the highest speed of the light-powered bacteria at high light intensity is about 10 to 11 \( \mu m/s \) (Fig. 2), which is smaller than that of the wild-type strain. Three strains share a similar body plan with the semi-major axis of the bacterial body \( a = 1.4 \pm 0.2 \mu m \) and the semi-minor axis \( b = 0.5 \pm 0.2 \mu m \) based on direct imaging of bacteria. The errors reported here are the standard errors, instead of the standard deviations, of the measurements.

All the three strains are cultured using a similar procedure. They are first cultured at 37.0°C with a shaking speed at 250 rpm for 14-16 hours in terrific broth (TB) culture medium [tryptone 1.2% (w/v), yeast extract 2.4% (w/v), and glycerol 0.4% (v/v)]. The saturated culture is then diluted 1:100 in TB culture medium and grown at 30°C for 6.5 hours. PR expression is triggered by 1 mM isopropyl \( \beta \)-D-thiogalactoside and 10 \( \mu M \) methanolic all-trans-retinal, which are supplemented in the mid-log phase for the synthesis of proteorhodopsin. The bacteria are harvested by gentle centrifugation (800g for 5 min) in the late log phase. After discarding the upper medium, we resuspend bacteria with motility buffer MB (0.01 M potassium phosphate, 0.067 M NaCl, 10 M EDTA, PH 7.0). The suspension is finally washed twice and adjusted to the target concentration. To create the mixture of tumblers and swimmers, we separately prepare suspensions of tumblers and swimmers both at the targeted concentration. We then mix the two suspensions at a predetermined ratio to control the fraction of active swimmers \( f \).

**2. Sample preparation and video microscopy**

For the wild-type bacteria, oxygen is necessary to maintain the swimming of bacteria. We deposit a 2 \( \mu l \) wild-type bacterial suspension on a microscope coverslip, which forms a free suspension-air interface on the top. The droplet is millimetric in the lateral directions (x-y) normal to the imaging plane and about 150 \( \mu m \) in height (z). The coverslip and the suspension are further enclosed in a humid chamber of \( \sim 1000 \, mm^3 \) to reduce evaporation as well as perturbation due to ambient air flows. An inverted microscope is used to image bacterial motions 70 \( \mu m \) above the coverslip, which shows bacterial flows in the 2D \( x-y \) plane inside the 3D suspension (SI Video 1).

For the light-powered bacteria, it is important to shut down the metabolic pathway of aerobic respiration, so that the locomotion of bacteria is solely controlled by the PR pump. We inject a suspension of light-powered bacteria into a sealed cell of 18 \( \times \) 3 \( \times \) 0.17 mm\(^3\). For concentrated bacterial suspensions above 35\( \% \), bacteria stop swimming after a few minutes in the cell due to the depletion of oxygen. Microscope illumination is then used to switch on/off and control the activity of the PR pump for bacterial swimming. 2D bacterial flows are imaged 80 \( \mu m \) above the bottom wall.

For most of our experiments, 2D flow fields are imaged through an inverted bright-field microscope using a 10\( \times \) (NA 0.3), 20\( \times \) (NA 0.5) or 40\( \times \) (NA 0.6) objectives. The field view of images ranges from 640 \( \times \) 640 \( \mu m^2 \) to 160 \( \times \) 160 \( \mu m^2 \). To measure the velocity orientational order and the flow energy in steady states, one-minute videos are taken 15 minutes after wild-type E. coli samples are loaded or 2 minutes after a new light condition is applied to light-powered E. coli samples. These times are sufficient for the sample to reach the steady state. Five-minute videos are taken for transition kinetics after light ramping. All the videos are recorded at 30 frames per second by a sCMOS camera. To control bacterial velocity, light intensity is tuned by the voltage of the light source. Three to twelve independent measurements are taken for each set of control parameters of our experiments.

To measure the dynamics of individual bacteria during the turbulent transition, we image fluorescent-labeled E. coli with an inverted fast confocal microscope using a 60\( \times \) objective (NA 1.4). The green fluorescent protein expressed in bacteria is excited by a 488 nm laser. The field view of the images is 180 \( \times \) 120 \( \mu m^2 \). Bacterial flows are imaged 10 \( \mu m \) above the coverslip. Five-minute videos are taken at 10 frames per second.
3. Image processing and data analysis

The velocity field of bacterial suspensions is obtained from raw videos using standard Particle Imaging Velocimetry (PIV). For each pair of neighboring frames, the interrogation window size shrinks from 19.6 × 19.6 µm² to 4.8 × 4.8 µm² in three iterations. The final lattice spacing of the velocity field is 2.4 µm. The velocity field in Fig. 1h shows the velocity vectors of every other lattice.

Energy spectrum quantifies the energy distribution over different length scales, \( \lambda = 2\pi/k \), where \( k \) is the wavenumber. To obtain the energy spectrum, we first calculate the Fourier transform of the 2D velocity field \( u_x(x, y) \) and \( u_y(x, y) \) to obtain \( U_k(x_k, y_k) \) and \( V_k(x_k, y_k) \). The point-wise kinetic energy density in \( k \)-space is then computed \( E(k_x, k_y) = (U_k(x_k, y_k)U_k^*(x_k, y_k) + V_k(x_k, y_k)V_k^*(x_k, y_k))/2 \), where * represents the complex conjugate. Finally, the energy spectrum \( E(k) \) is obtained by summing up \( E(k_x, k_y) \) at a constant \( k = (k_x^2 + k_y^2)^{1/2} \). An alternative way to calculate \( E(k) \) is through the Fourier transform of the two-point velocity correlation function \( \langle \vec{v}(r_0) \cdot \vec{v}(r_0 + \vec{r}) \rangle/r_0 \), which yields quantitatively similar results.

To extract bacterial nematic order, we perform Fast Fourier transformation (FFT) on the local regions (20 × 20 µm²) of confocal images. The FFT patterns are first normalized to the local nematic order parameter of bacteria \( S = \sum_j (\cos^2 \alpha_j - 1)/2 \) within the range of our experiments (Fig. S4 in [44]), where \( \alpha_j \) is the angle between the orientation of bacterium \( j \) with respect to the mean orientation of all the \( N \) bacteria in the local region in 3D.

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