

Collective motion and density fluctuations in bacterial colonies

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Flocking birds, fish schools, and insect swarms are familiar examples of collective motion that plays a role in a range of problems, such as spreading of diseases. Models have provided a qualitative understanding of the collective motion, but progress has been hindered by the lack of detailed experimental data. Here we report simultaneous measurements of the positions, velocities, and orientations as a function of time for up to a thousand wild-type *Bacillus subtilis* bacteria in a colony. The bacteria spontaneously form closely packed dynamic clusters within which they move cooperatively. The number of bacteria in a cluster exhibits a power-law distribution truncated by an exponential tail. The probability of finding clusters with large numbers of bacteria grows markedly as the bacterial density increases. The number of bacteria per unit area exhibits fluctuations far larger than those for populations in thermal equilibrium. Such "giant number fluctuations" have been found in models and in experiments on inert systems but not observed previously in a biological system. Our results demonstrate that bacteria are an excellent system to study the general phenomenon of collective motion.

swarming | self-organization | active suspensions

Despite differences in the length scales and the cognitive abilities of constituent individuals, collective motion in systems as diverse as bird flocks, mammal herds, swarming bacteria, and vibrating granular particles (1–8) produces similar patterns of extended spatiotemporal coherence, suggesting general principles of collective motion. One approach to unveil these principles has been to model individuals as interacting self-propelled particles, which align their motions with neighbors (8–13). Some models also include repulsive and attractive interactions between particles in addition to the local alignment of velocities. With empirically chosen parameters such as the range over which the local alignment occurs, self-propelled particle models produce motions qualitatively similar to the observations. For example, within certain parameter regimes, models (8, 13) predict that collectively moving individuals form dynamic clusters, as often seen in fish schools or mammal herds (1); these clusters lead to large fluctuations in population density. Quantitatively, analytic theories based on liquid crystal physics (14–16) have predicted that these density fluctuations should scale with system size differently from fluctuations in thermal equilibrium systems.

In contrast to numerical models and analytical theories, quantitative experiments have been limited (2–8), though decisive experiments are urgently needed to test the theoretical assumptions, determine sensitive modeling parameters, and verify theoretical predictions. The lack of experimental data is mainly due to technical difficulties. In conventional macroscopic systems, such as bird flocks, it is exceedingly challenging to track individual motions of a large population over long periods of time, and studies with systematic parameter variation are rarely possible.

Recent studies (17–22) have shown that concentrated swimming bacteria, which can be considered as self-propelled polar microrods, under well-controlled conditions exhibit collective motion. These studies suggest that bacterial systems can serve as a promising alternative to the macroscopic systems to investi-

gate general phenomena of collective motion. However, in previous studies, bacteria were so closely packed that it has not been possible to identify and simultaneously track many individual bacteria in collective motion for a long period of time.

In this article, we investigate collective bacterial motion in wild-type *Bacillus subtilis* colonies growing on agar substrates. Inside colonies, bacteria swim in a micrometer-thick film of liquid on the agar surface (23, 24); the bacteria are not observed to swim over one another. By tracking many individual bacteria, we quantify the correlations among bacteria, statistical properties of dynamic clusters, and the scaling of density fluctuations.

Results

Spatial Correlations. Movies of bacterial motion are recorded at various bacterial densities. Each movie contains 6,000 consecutive images in which bacteria appear as white elongated objects. From the movies, we extract center-of-mass \vec{r}_i , instantaneous velocity \vec{v}_i , and cell body orientation \hat{y}_i for 95% of all bacteria in the imaging window ($A = 90 \times 90 \mu\text{m}^2$). Typical instantaneous configurations at two densities are shown in Fig. 1.

Bacteria aggregate in clusters and move collectively as shown in Fig. 1 (also [Movies S1](#) and [S2](#)). To quantify the local correlation of individual motions, we compute spatial correlation functions (18–20, 25–27) in a "local coordinate frame" with axes along (\hat{y}_i) and perpendicular (\hat{x}_i) to the cell body, as defined in the inset of Fig. 1A. The two-dimensional correlation functions, defined in *Materials and Methods*, for two densities are shown in Fig. 2A–F, and their transverse profiles along $y = 0$ are shown in Fig. 2G–I.

The pair correlation function $g(x,y)$ quantifies the probability per unit area (normalized by the area density ρ) of finding another bacterium at the location (x,y) away from the reference bacterium. The transverse profiles of the pair correlation function $g(x,y=0)$ in Fig. 2G show strong peaks at $x = 1.44 \mu\text{m}$ for $N_{\text{total}} = 343$ and $x = 1.08 \mu\text{m}$ for $N_{\text{total}} = 718$; thus bacteria in a cluster are positioned very close to a nearest neighbor. Peaks corresponding to the second and third nearest neighbors are also discernible for $N_{\text{total}} = 718$. As shown in Fig. 2A and D, such bacterial aggregation in the flank extends in the longitudinal direction up to $y \approx 3.8 \mu\text{m}$, which is roughly the length of the excluded volume. Close neighbors have similar orientation and velocity as the reference bacterium, as evidenced by the high correlation in orientation (Fig. 1B, E, and H) and velocity (Fig. 1C, F, and I). The positional, velocity, and orientational correlations are evident, for example, in the cluster at $(20 \mu\text{m}, 30 \mu\text{m})$ in Fig. 1B.

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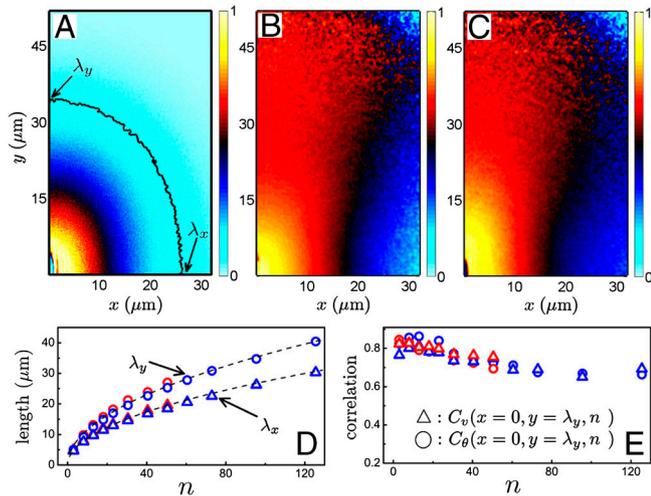


Fig. 3. Statistical properties of dynamic clusters. (A) Cluster-size-dependent pair correlation $g(x,y,n)$, (B) orientational correlation $C_\theta(x,y,n)$, and (C) velocity correlation $C_v(x,y,n)$ functions. In A, the black line is the contour for $g(x,y,n) = 0.1$, which intersects the x and y axes at $(\lambda_x, 0)$ and $(0, \lambda_y)$. Longitudinal λ_y and transverse λ_x lengthscales are plotted in D as functions of the cluster size n . The black dashed lines in D are fits of $\lambda \propto n^{0.5}$. In E, values of velocity and orientational correlation at $(0, \lambda_y)$ are plotted against n . In D and E, data in blue and red are from $N_{\text{total}} = 718$ and $N_{\text{total}} = 513$, respectively. More cluster-size-dependent correlation functions computed at various conditions can be found in Figs. S2 and S3B.

as $n^{0.5}$, which means the packing density within a cluster depends weakly on its size. As N_{total} increases from 513 to 718, the physical dimensions of a cluster with a given size decrease slightly; this is consistent with the shift of peaks in pair correlation functions (Fig. 2G). Cluster-size-dependent velocity $C_v(x,y,n)$ and orientation $C_\theta(x,y,n)$ correlation functions show a high level of coherence within a cluster. Their numerical values at a point on the edge of clusters, plotted in Fig. 3E, decrease slightly from 0.85 to 0.7 as n increases from 3 to 125.5. Furthermore, correlation functions computed in clusters of the same size exhibit little dependence on N_{total} , as shown by data with different colors in Fig. 3E.

The probability to find a cluster with size n is described by the cluster size distribution function, $P(n)$, as shown in Fig. 4 for three density conditions. $P(n)$ first decays as a power law (see Inset), then evolves into an exponential tail (main plot). Quantitatively, $P(n)$ is well described by

$$P(n) = An^{-b}e^{-n/n_c}, \quad [1]$$

where the cutoff size n_c characterizes the transition from power law to exponential behavior. We treat b and n_c as fitting parameters, and prefactor A is determined by a normalization condition: $\sum_n P(n) = 1$, where the summation over n runs from 1 to the size of the largest cluster observed. We find that the exponent b is independent of bacterial density ($b = 1.85$), whereas n_c increases with bacterial density, $n_c = 6.5$ for $N_{\text{total}} = 343$ and $n_c = 75$ for $N_{\text{total}} = 718$. The rapid increase of the cutoff size n_c with N_{total} means that the probability of finding large clusters grows markedly as the density of bacteria increases, which is presumably the reason for the larger correlation length in Fig. 1 for $N_{\text{total}} = 718$. The prefactor A decreases from $A = 0.75$ for $N_{\text{total}} = 343$ to $A = 0.57$ for $N_{\text{total}} = 718$; the change in A value is small enough that we can rescale and collapse all data onto a single curve as shown in the inset of Fig. 4. Beyond our system, the cluster size distribution described by Eq. 1 has been observed in fish schools and buffalo herds (8, 36, 37), which suggests general principles of collective motion across many length scales.

Density Fluctuations. Bacteria in clusters are closely packed, which leads to high local density. Clusters are mobile and they often leave empty space in regions they just pass; this leads to low density in those regions. Consequently, mobile clusters cause large density fluctuations, as shown by a temporal record of the total number of bacteria in the whole imaging window in Fig. 5A. This record exhibits a maximum about twice as large as the minimum and has a standard deviation $\Delta N = 71$ about 10% of the mean. Besides being large in amplitude, these density fluctuations scale with the mean differently from fluctuations in systems in thermodynamic equilibrium (7, 12–14, 16, 35, 38). For systems in thermal equilibrium where fluctuations obey the central limit theorem, ΔN is proportional to \sqrt{N} ; therefore $\Delta N/\sqrt{N}$ should be a constant for all N . However, Fig. 5B shows that, for $N_{\text{total}} = 343$, where the cluster sizes remain small, $\Delta N/\sqrt{N}$ initially increases, then saturates. For higher density ($N_{\text{total}} = 718$), the data show anomalous density fluctuations, sometimes called giant number fluctuations (7, 13). Here, the standard deviation ΔN grows more rapidly than \sqrt{N} and scales as $\Delta N \propto N^{0.75 \pm 0.03}$. Such anomalous density fluctuations have been observed in a numerical model of self-propelled polar particles that move unidirectionally like bacteria but interact differently according to phenomenological rules (13). The scaling exponent 0.8 found in the simulations is close to 0.75 ± 0.03 measured for bacteria, which suggests that general statistical properties of collective motion might be independent of the details of microscopic interactions. Systems of apolar (bidirectionally moving) particles also exhibit anomalous density fluctuations but with a scaling exponent closer to 1, as found in theory (16), numerics (12), and experiments (7, 38). Thus the scaling exponent seems to depend on the mode of motion of the individual particles.

Discussion

Though bacteria have no central nervous system and are orders of magnitude smaller in physical dimension than macroscopic animals such as birds or fish (1–6), they locally align their individual motions like their macroscopic counterparts. Local alignment, which can arise from different interactions in different systems, is the essential ingredient responsible for collective motion according to models (8–13) and theories (14–16) designed to capture general principles of collective motion in a wide range of systems. Locally aligned bacteria move collectively in dynamic clusters with distinct statistical properties. These clusters lead to large fluctuations in population density, which exhibit an anomalous scaling with system sizes.

Our results demonstrate that bacteria are an excellent model system for studying general principles of collective motion. Their

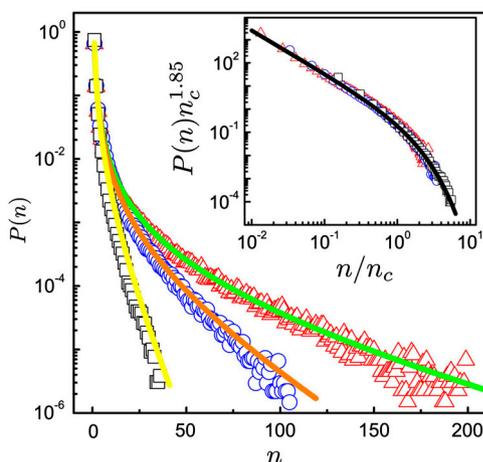


Fig. 4. Size distribution of bacterial clusters for three bacterial densities: $N_{\text{total}} = 343$ (squares), $N_{\text{total}} = 539$ (circles), and $N_{\text{total}} = 718$ (triangles). Solid lines are fits to Eq. 1. (Inset) All data collapse onto a master curve by rescaling and are plotted in a log-log frame with a solid line showing the rescaled fit.

dependent correlation functions (for $81 \leq n \leq 110$) computed for different parameters in Fig. S3B also exhibit little difference. Lower values of R_d and A_d naturally lead to slightly higher correlations for velocity and orientation (first row in Fig. S3B).

Definition of Spatial Correlation Functions. The two-dimensional pair correlation is defined as

$$g(x,y) = \frac{1}{\rho} \left\langle \sum_{j \neq i} \delta[x\hat{x}_i + y\hat{y}_i - (\vec{r}_i - \vec{r}_j)] \right\rangle_i, \quad [2]$$

where δ is a Dirac function, $\rho = N_{\text{total}}/A$ is the area density, $\langle \dots \rangle_i$ represents average over all reference bacteria i , and the position difference $\Delta \vec{r} = x\hat{x}_i + y\hat{y}_i$ is expressed in i th local frame, as defined in the inset of Fig. 1A. The orientation correlation function and velocity correlation function are defined as

$$C_\theta(x,y) = \langle (\hat{y}_i \cdot \hat{y}_j) \delta[x\hat{x}_i + y\hat{y}_i - (\vec{r}_i - \vec{r}_j)] \rangle_{ij} \quad [3]$$

and

$$C_v(x,y) = \frac{\langle (\vec{v}_i \cdot \vec{v}_j) \delta[x\hat{x}_i + y\hat{y}_i - (\vec{r}_i - \vec{r}_j)] \rangle_{ij}}{\langle \vec{v}_i \cdot \vec{v}_i \rangle_i}, \quad [4]$$

where $\langle \dots \rangle_{ij}$ represents average over all possible pairs.

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