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# Hydrodynamic Interactions, Hidden Order and Emergent Collective Behavior in an Active Bacterial Suspension

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Spontaneous self-organization (clustering) in magnetically oriented bacteria arises from attractive pairwise hydrodynamics, which are directly determined through experiment and corroborated by a simple analytical model. Lossless compression algorithms are used to identify the onset of many-body self-organization as a function of experimental tuning parameters. Cluster growth is governed by the interplay between hydrodynamic attraction and magnetic dipole repulsion, leading to logarithmic time dependence of the cluster size. The dynamics of these complex far-from-equilibrium structures are relevant to broader phenomena in condensed matter, statistical mechanics and biology.

One of the distinguishing characteristics of biological systems is the emergence of order from the interactions of discrete active components operating far from equilibrium. Insights into how these interacting components produce functional structures across many length scales is of fundamental importance in biological systems, from collections of eukaryotic cells[1] and bacteria[2], to ant colonies [3] and bird flocks[4]; the phenomena extend to non-biological active matter such as colloids[5, 6] and macroscopic robot swarms [7]. A complete description of these systems requires not only an understanding of the microscopic interactions between components, but also the principles governing the dynamics of the many-body states. In this letter, we present an experimental and theoretical analysis of both the microscopic hydrodynamic interactions and onset of emergent many-body self-organization, in a prototypical active matter system [8, 9] - a suspension of motile bacteria.

The bacterial species selected for this study (*Magnetotacticum magneticum* AMB-1[10]) is chosen for its innate magnetism, which renders it amenable to direct external control [11–15], allowing systematic imposition of orientational coherence on the population. Furthermore, the species is motile - the chemically powered flagellum (a thin helical appendage responsible for cellular propulsion) provides the source of activity. This attribute gives rise to attractive hydrodynamic interactions between cells that produce self-organized states (clusters).

To quantify the pairwise hydrodynamic interactions between two cells, a dilute suspension of AMB-1 is placed in a fluid cell and subjected to an external field ( $H_z < 100G$ ) oriented perpendicular to the surface, using a previously described system [15, 16]. As the cells encounter the surface, they either swim in circular planar orbits stabilized by hydrodynamic effects[17], or align normal to the surface and execute a lateral random walk (in a magnetically stabilized state)[15]. Pairs of cells in this latter state experience attractive hydrodynamic interactions and rotate about their center of mass (Fig. 1 c, e,

inset and the supplemental videos [18]). To determine the spatial dependence of these interactions, a pair of AMB-1 cells is isolated in the fluid cell, far from other cells. The pair is perpendicularly oriented under constant  $H_z$  (Fig. 1a) causing them to approach one another and form a stably rotating doublet. Once the cells have been brought together, the fields are removed (randomizing cell positions) or tilted (navigating cells along diverging paths [15]). When separated by the desired distance  $H_z$  is reapplied and the flow field mediated intercellular interactions return the cells to close contact. This process is repeated until a number (87) of trajectories are compiled, yielding the time-of-flight ( $\Delta t$ ) for the cells to arrive in the doublet configuration (close contact) and angular velocity ( $\omega$ ) as a function of intercellular distance ( $r$ ), as shown in Fig. 1 c,e.

We attribute the attractive interaction between cells to Stokeslet ( $1/r$ ) [19] dominated flow fields (the flow arising from a point force) associated with the flagellum. Freely swimming organisms at low Reynolds number ( $Re$ ) are generally considered to be force- and torque-free [20]. Hence, the leading order term in the multi-pole expansion of the flow field is taken to be a Stokeslet dipole. However, a power series fit suggests the presence of lower order terms (Fig. 1c), implying that an unpaired force monopole  $f_m$  acts on the fluid. The force free condition is then maintained by the balance of the flagellar thrust by the surface. We therefore model the cell's flow field using a singularity system comprising a Stokeslet along with its image system (accounting for the surface [21] Fig 1b). The radial component of the flow velocity  $u_r(r)$  in the plane of the singularity, which is assumed to be proportional to the force between the cells, is given by,

$$u_r(r) = -\frac{f_m}{8\pi\eta} \frac{12h^3r}{(4h^2 + r^2)^{5/2}} = \frac{1}{2} \frac{dr}{dt} \quad (1)$$

where  $h$  the height of the singularity;  $dr/dt$  is the rate of change of the intercellular separation and  $\eta$  the dynamic viscosity of water. The time  $\Delta t$  required to travel

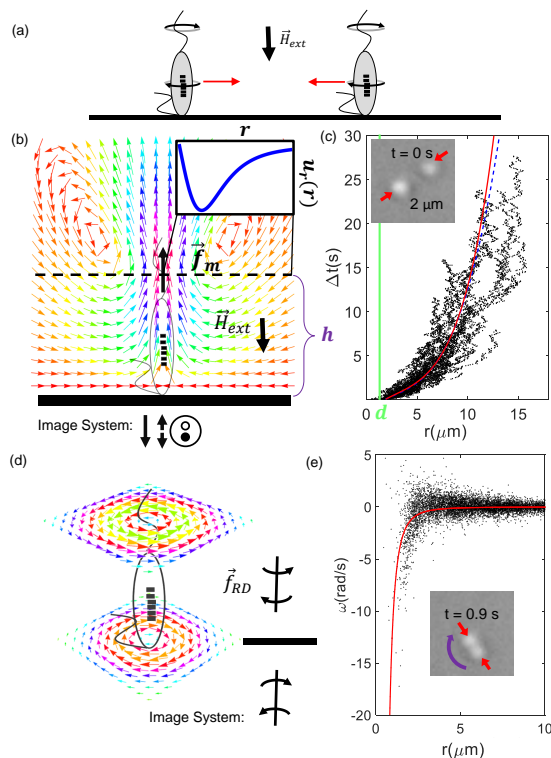


FIG. 1. a) schematic of oriented cells and resulting hydrodynamic interaction. b) Flow field of a pure Stokeslet at height  $h$  from a surface with image system (force monopole, force dipole, source dipole). Inset, profile of the velocity (Eq. 1) derived from the flow field along the dashed line at height  $h$  above surface. c) time of flight ( $t$ ) vs intercellular spacing ( $r$ ) for 87 cellular trajectories from three distinct pairs of cells, along with fit to model (in red) and power series (blue) revealing presence of lower order ( $1/r$ ) terms. Vertical line indicates the minimum cell separation  $d$  from fit. Inset, microscopy images of cells forming a rotating doublet. d) Flow field of a rotlet dipole and image system, along with the singularities. e) the angular velocity ( $\omega$ ) vs  $r$  with fit to rotlet dipole ( $1/r^3$ ) model (red).

from an initial separation  $r$  to a final closest contact distance  $d$  is determined by integrating Eq. (1). Least-squares fits to a running average of the data show good agreement and yield reasonable estimates for the three parameters,  $f_m$ ,  $d$  and  $h$ . In particular, a singularity height of  $h = 2.6 \pm 0.1 \mu\text{m}$  (comparable to average cell length  $\sim 3 \mu\text{m}$ [22]), a minimum intercellular spacing of  $d = 1.8 \pm 0.2 \mu\text{m}$  (agrees with optical image of doublet) and a flagellar thrust  $f_m = 0.20 \pm 0.02$  pN (consistent with previous measurements of hydrodynamically analogous species [23–25]) are obtained.

The source of the rotational motion is ascribed to a rotlet dipole field ( $\sim 1/r^3$ ) emanating from the rotating flagellum and counterrotating body [21] (Fig. 1d). The measured angular velocity of the cells about their center of mass along with a fit to a rotlet dipole flow field model (Fig. 1e) shows good agreement. The observed

clockwise rotation (viewed from above) for all intercellular separations  $r$  implies the net rotation of the cell pair is dominated by coupling of the cell body (rather than the flagellum) to the rotlet dipole field. The body has a larger drag coefficient and hence couples more strongly to flow than the flagellum leading to unidirectional motion. The rotation of the flagellum merely attenuates the net effect (rotlet dipole flow field decays as  $\omega \sim 1/r^3$ , relative to rotlet field  $\omega \sim 1/r^2$ ).

In the many-body case, the attractive hydrodynamic forces result in the formation of clusters, which continually rearrange and rotate under the influence of the rotational effects (Fig. S1a and the supplemental video [26]), analogous to previous observations of spontaneously oriented non-magnetic bacteria [27, 28]. The effect of these rotational flows, along with the packing problems created by the spirochete AMB-1 cell morphology[10], prevent the onset of crystalline order within the cluster. Numerical simulation of the dynamics based on an Euler method, including a Stokeslet derived interaction with a screening cutoff and a stochastic force accounting for Brownian and biogenic noise displays good agreement with the experimental results [29].

To quantify the self-organization, a Lempel-Ziv compression algorithm [30] is applied to each recorded image. As recently demonstrated [31], this algorithm, which is widely used in file compression applications, yields the computable information density (CID),  $f_c$ , which bounds the Shannon entropy [31] and is defined as

$$f_c \equiv \frac{\mathcal{L}(x)}{L} \quad (2)$$

where  $\mathcal{L}(x)$  is the length of a losslessly compressed string  $x$  and  $L$  is its uncompressed length. For non-equilibrium processes, traditional methods for characterizing order-disorder transitions often fail, and in many instances, the order parameters are unknown. Nonetheless, the CID provides a generic measure of the order in the system without knowledge of the particular nature of the ordering. Simply compressing each successive microscopy image and recording the file size allows the determination of the experimental conditions under which the information entropy drops in time. This allows a phase boundary to be constructed without explicitly identifying an order parameter.

To define the onset of self-organization, a pulsed field sweep is conducted. Fig. 2 illustrates self-organization for a suspension observed in wide field (20x objective, see Supplemental videos [32]). At  $t = 0$ , the field ( $H_z = 10 - 100G$ ) is turned on resulting in an initial rise in  $f_c$ . This transient ( $\sim 1$ -2 seconds) results from the rapid increase in the number of oriented cells swimming towards the surface, as well as the finite re-orientation time of cells already at the surface upon application of  $H_z$ . Once the cell density stabilizes ( $\sim 2.5s$  after  $H_z$  is introduced),  $f_c$  begins to decrease as self-organization pro-

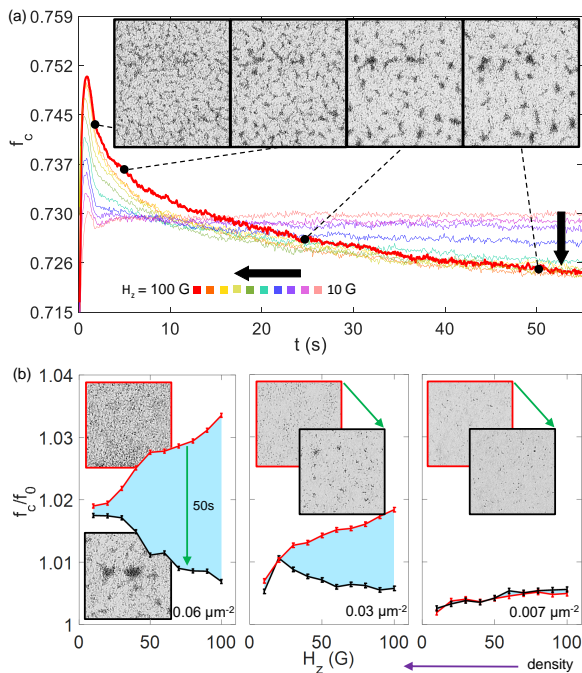


FIG. 2. a)  $f_c(t)$  for a population subjected to 10 – 100G fields for 55 seconds (bold arrow indicates increasing magnetic field). The bolder, red curve shows the most dramatic drop in  $f_c$  under a 100G field. As the field is decreased (other colors), the extent of the decay in  $f_c$  is reduced until at low values it remains nearly static in time. inset) selected microscopy images from the self-organization process associated with the red (100G) curve, taken at  $t = 2.5, 5, 25$  and  $50$  s. b) initial (red) and final (black) values of the scaled CID ( $f_c/f_0$ ) as a function of field strength for various densities. As density decreases (left to right), the self-organization disappears at all field strengths (as seen in the shaded area) Inset, selected images from 60G field pulse showing initial (red outline) and final (black outline) representative configurations.

ceeds. Fig. 2a illustrates  $f_c(t)$  for a sequence of 10 field strengths (at roughly constant density), each beginning from a random cell configuration. As the field strength is decreased, the decay of  $f_c$  with time is reduced until no significant reduction occurs over the interval. The gradual attenuation of the decay (Fig. 3a) indicates the disappearance of order as the magnetic field is reduced. It is noted that the structures themselves undergo dramatic qualitative changes ranging from uniform density ( $t = 0$ s), filamentary networks ( $t = 2.5, 5.0$ s) and isolated high density islands ( $t = 25, 50$ s), for the high field (100 G) example depicted in Fig. 3 a,inset.

To determine the effect of cell density on self-organization, pulsed field sweep (10G - 100G) experiments were repeated over a range of densities. Between each field sweep,  $H_z$  is removed, allowing the cells to orientationally decohere and randomize their positions. Because cells swim freely in the zero-field state, a small fraction move out of the field of observation due to aerotaxis,

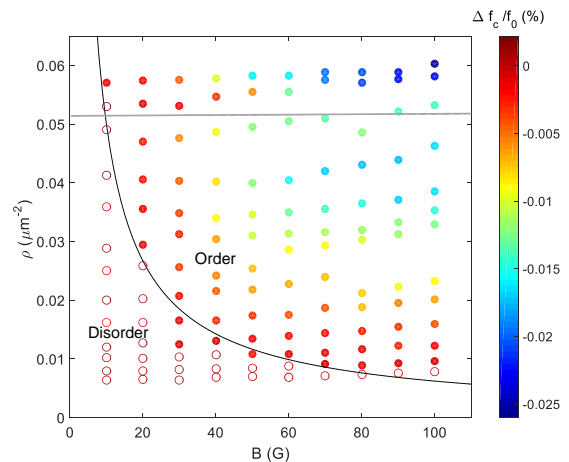


FIG. 3. Phase diagram illustrating the boundary between clustered states (closed circles) and disordered states (open circles). The color indicates the percentage drop in the scaled CID ( $f_c/f_0$ ). The black line is a approximate power law fit to the phase boundary. The gray line indicates the threshold beyond which the density may not be reliably determined optically. Hence for these points the recorded density should be interpreted as a lower bound.

leading to a continual reduction in the density ( $\rho$ ). To track this decay,  $\rho$  is calculated directly from images by counting the cells (before each application of  $H_z$ ), using ImageJ [33]. When the mean intercellular spacing in this disordered state approaches the optical size of the cells, this method no longer reliably measures the density at the surface and hence merely provides a lower bound (see Fig. 3).

To determine the presence of order at a given  $\rho$  and  $H_z$ , the change in  $f_c$  over a fixed time interval (55 s) is calculated. To compensate for density fluctuations within the interval, the initial and final values of  $f_c$  are respectively scaled by the zero-field CID ( $f_0$ ), collected before and after application of the field. If the scaled value ( $f_c/f_0$ ) drops more than the width of the noise in  $f_c/f_0$  during the time the field is applied, we infer the presence of order.

Fig. 2b shows the initial (red) scaled CID ( $f_c/f_0$ ) plotted alongside its final (black) value for several field sweeps at different densities. Fig. 2b (left) is a high density case in which  $f_c/f_0$  drops (shaded region) more than the noise, at all measured field strengths. At a slightly lower density, Fig 2b (center), the onset of self-organization only occurs when clear separation in  $f_c/f_0$  is first evident at  $H_z > 20$  G. As the density is further reduced, the order disappears entirely (as evidenced in the shaded area) at even the highest magnetic field strengths (Fig. 2b, right) indicating a low density of oriented cells.

Fig. 3 summarizes results of 13 field sweeps conducted with the population corresponding to Fig. 2, which allows for the construction of a phase boundary separating

ordered (open circles) from disordered states (closed circles). The color bar indicates the percentage drop in  $f_c/f_0$  over the 55 s interval. Order disappears as the orientational coherence across the population is destroyed when  $H_z$  approaches zero. This decoherence occurs by: (i) the interplay between surface-induced hydrodynamic torques and the magnetic interactions that result in planar swimming [15, 17] and (ii) increase in the orientational noise [22]. Further, as  $\rho$  is reduced, stochastic forces begin to dominate the coherent hydrodynamic interactions when the timescale for attractive interaction becomes comparable to that of cell diffusion. As a result, the cells fail to attain an ordered state.

To understand the kinetics of the clustering process, the time evolution of the radial distribution function  $g(r)$  (Fig. 4) is computed from the microscopy images. Initially ( $t \sim 0.1s$ )  $g(r)$  is largely flat, apart from a cut off associated with the cell size. As clustering proceeds, a peak associated with cell-cell close packing separation distance grows and widens in time. The width of the peak is associated with the largest cluster dimension ( $\sim 20\mu m$ ), while its area is proportional to the fraction of cells in the clustered state. An additional broad peak centered at an increasing separation distance is associated with the mean cluster-cluster distance. The probability of finding cells separated by larger distances ( $> 100\mu m$ ) is repressed relative to a random distribution (dashed line), corresponding with the distance between clusters and voids of reduced density.

Fig. 4, inset, shows the growth of the primary peak ( $r \sim 5\mu m$ ) area  $I(t)$ , relative to the area at the initial time  $I_0$ . After a transient ( $\sim 1s$ ), the growth of the  $I(t)$ , and hence the size of the clusters, scales logarithmically in time. It has been previously shown both theoretically and experimentally that Brownian coalescence of monopolarly charged suspensions leads to logarithmic time dependence [34–36]. In these systems, particles come into contact through random collisions and stick through short-range contact forces (e.g. a van der Waals interaction). As the clusters accumulate charge, repulsion begins to suppress the growth rate. Similarly, in the present system, attractive hydrodynamic interactions are opposed by magnetic dipole-dipole repulsion. While the hydrodynamic attraction and magnetic repulsion are both predicted to have the same long range asymptotic behavior ( $\sim 1/r^4$ ), the hydrodynamic effects experience an effective cutoff, due to screening and stochastic effects. The hydrodynamic forces are of greater strength at  $\mu m$  range ( $\sim pN$ ) relative to the magnetostatic forces ( $\sim 0.01pN$ , for magnetic moments  $\sim 10^{-16}Am^2$  [22]) for pairs of cells. However, as the clusters grow and increase in total magnetic moment, the magnetic forces become comparable to the hydrodynamic forces. Thus, the structure and logarithmic kinetics of the clusters may be understood as an interplay between attractive hydrodynamics with a finite range and repulsive magnetic inter-

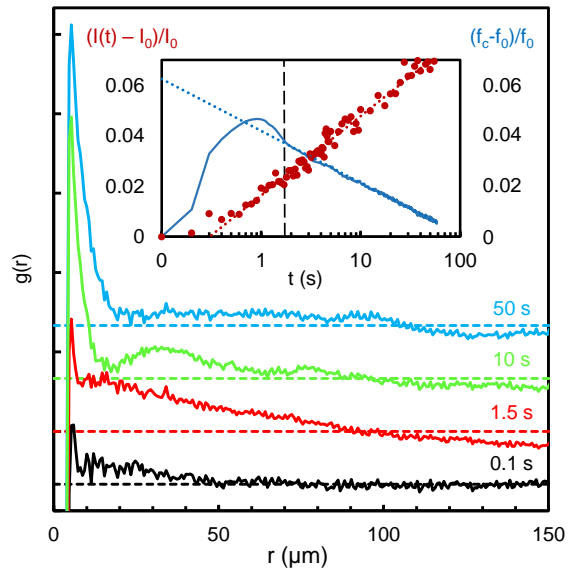


FIG. 4.  $g(r)$ , offset for clarity at  $t = 0.1, 1.5, 10,$  and  $50$  seconds after application of a  $100$  Oe external field (corresponding with the images in Fig. 3,a). The dashed line corresponds with the value of  $g(r)$  expected for a non-interacting gas (a flat distribution). Inset, shows a logarithmic increase in the peak associated with clustered cells (red) and the logarithmic scaling of the CID (blue) in time. Vertical dashed line indicates the onset of logarithmic scaling at  $t \sim 2s$  after the initial transient .

actions that scale with the cluster size, thereby inhibiting the continued rapid growth of the clusters. In this sense, the process may be understood as an active matter analog to the self-focusing regime in passive charged colloids [34]. Interestingly, as shown in Fig. 4, inset, the CID also scales logarithmically in time after an initial transient ( $t \sim 1s$ ). This suggests a direct physical interpretation of the CID as providing information about the configurational entropy of the cells, which decreases as they coalesce, and are constrained to occupy a smaller volume.

In conclusion, we have shown that when oriented near a surface, AMB-1 experience attractive hydrodynamic interactions arising from their flagellar activity which are well captured by a simple analytical model based on a pure Stokeslet and its image system. Moreover, these interactions along with dipolar magnetic repulsion, give rise to spontaneous, self-organized bacterial clusters where the CID (which bounds the Shannon entropy), reveals the phase boundary defining the onset of self-organization. Kinetics of cluster growth are governed by the interplay between hydrodynamic attractive forces and magnetic repulsion, analogous to self-focusing of charged inactive colloids. Taking advantage of the high degree of experimental control and theoretical tractability of the present system, future studies should

address broad questions in non-equilibrium active self-organization. Particularly salient are questions regarding the specific nature of the ordering and what critical behavior, if any, accompanies the onset of self-organization. Additionally, the thermodynamic implications of the relationship between dissipation and structure formation in active systems should be explored.

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