Modulated Collective Behaviors and Condensation of Bacteria

Mei Mei Bao,†a Isaiah Eze Igwe,b Kang Chen,*a Lei Wang,a and Tian Hui Zhang**a

Bacteria can spontaneously develop collective motions by aligning their motions in dense systems. Here, we show that bacteria can also respond collectively to an alternating electrical field and form active clusters. The active clusters can diffuse and merge. Most intriguingly, as the active clusters go beyond a critical size, they split into smaller ones spontaneously. The critical size for splitting depends on the frequency of electric field and the concentration of bacteria. As the formation of active clusters can be explained by the electric-field-induced long-range hydrodynamic attraction, we suggest that the critical size and the spontaneous splitting result from the instability of the core-shell structure due to the inhomogeneous stress inside the active clusters. The findings in this study illustrate that bio-systems can respond collectively to an external field, promising an effective way to control and modulate the behavior of organisms. Moreover, the controlled aggregation and condensation of bacteria offer a robust approach to improve the local concentration of bacteria for early and rapid detection, which has wide applications in clinical applications.

Introduction

Active matters are able to take up energy from their environment and self-organize into various collective motions. To understand the general mechanisms behind collective motions, many studies have been conducted in colonies of bacteria1, 2, fish school3 and bird flocks4. Vicsek et al suggested that collective motions are ubiquitous in systems with the mechanism of velocity alignment5. Experimentally, the aligning mechanism may arise from hydrodynamic interaction6, 7, 8, 9, 10, 11, 12 or steric interaction between active units13, 14. In suspensions of Escherichia coli or Bacillus subtilis where the long-range hydrodynamic interactions offer the aligning mechanism, collective motions including flocking and rotating vortices have been observed and studied extensively15, 16, 17. As hydrodynamic flows are directed or modulated by external confinements, collective motions can be modulated18, 19, 20, 21, 22, 23, 24, 25. For example, as dense suspensions of Bacillus subtilis are confined in a long and narrow macroscopic ‘racetrack’ channel, spiral vortices emerge26. In circular channels, the geometrical confinement on flows leads to a directed fluid flows which produce arrays of counter-rotating vortices.

In suspensions of magnetotactic bacteria (MTB), the collective behaviors can be tuned and modified by an external magnetic field due to the interplay between the hydrodynamic interactions and the field-induced interactions27, 28. For example, as a uniform magnetic field is applied into the suspension, the motion of MTB becomes well-directed along the field29. As they are confined in droplets, vortex and patterns can form30, 31. These examples demonstrate that the combination of hydrodynamic interactions and the field-induced interactions can produce much more complex collective behaviors.

However, magnetic field is applicable only for magnetotactic bacteria. As a popular property, bacteria, such as E. coli, are generally negatively charged in suspension32, 33. It is expected that the coupling between hydrodynamics and an electric field may impose additional control on the behavior of individual bacteria and thus their collective behavior.

As an effective controlling method, electric fields have been employed in separating and trapping cells. For example, blood cells characterized by different dielectrophoretic properties can be separated and selected by an electric field34, 35. As the electric field is spatially patterned, cells can be trapped and forms patterned distribution36. Despite of its wide interest, however, the effect of electric field on collective motions of bacterial has not been explored. Well-controlled emergence of collective motions is of great importance in understanding the general principles of self-organizations. Here, in this study, the collective behaviors of E.coli bacteria are investigated under an alternating current (AC) field. It is found that the E. coli bacteria can respond collectively to a low-frequency field and form active clusters. The radius of active clusters oscillates with the field at the same frequency. They diffuse and merge into larger ones as they meet. However, the size of the cluster is self-limited: as the radius of the clusters exceeds a critical size, they split into smaller ones. The field-induced reversible formation of oscillating active clusters demonstrates a new type of collective behaviors of active matter. Moreover, the formation of active clusters of bacteria significantly improves the local concentration of bacteria. This is of great importance in microbiological diagnosis. Bacterial infections are currently a major cause of fatal disease in humans. Rapid and accurate detection of bacteria is the key to preventing large-scale infectious diseases37. Traditional diagnosis and characterization techniques need to incubate bacterial specimens from hours to days to increase concentration up to detectable levels, which is time-consuming. The reversible aggregation and condensation of active bacteria observed in this study provide a rapid and robust approach to improve the local
concentration of bacteria, which has potential clinical applications, such as early and rapid detection.

Experiment Methods

*E. coli* bacteria are employed in this study. The bacterial cell has a rod-like body which is characterized by a length of ~2.0 \( \mu m \) and a diameter of 0.8 \( \mu m \). They swim by rotating flagella to produce a backward flow\(^{16, 40} \). Bacterial cultures were prepared with the protocol as that described in Ref.\(^{34} \). The bacterial samples were washed three times by centrifugation to remove the culture medium. Bacterial cells were then dispersed in deionized water by sonication. The suspension of bacteria is then injected to a chamber constructed by two conducting indium-tin-oxide (ITO) coated glasses which are separated by insulating separators (Fig.1a). The height of the insulating separator is around 180 \( \mu m \). The dynamic processes were observed by a Nikon microscope with the 40x objective and recorded by a CMOS camera (CB019MG-LX-X8G3) at a rate of 10 frames per second.

Results

To avoid directed motions along the field \( E \), an alternating electric field (AEF) is employed (Fig.1a). Observations were conducted with a concentration of 2.4x10\(^8 \) cfu/ml (colony formation unit, indicating the total number of bacterial communities per milliliter) which is far below the critical concentration for flocking and swarming\(^3, 41 \) such that no collective motions can occur spontaneously in the absence of an electric field. Before the application of AEF, bacterial swim randomly and distribute uniformly. When an electric field \( E \) higher than 0.05 V/\( \mu m \) is applied, bacterial cells were absorbed quickly to the electrodes and got damaged there. For AEF with a frequency above 100 Hz, no response and aggregation of bacteria were observed. As the frequency is below 100Hz, a dense phase forms in the system. However, collective motions are absent for frequency above 10 Hz. When the system is subjected to a field with frequency below 1.0 Hz, oscillating clusters emerge.

Figure 1b-d presents a typical dynamics process observed at the AEF of 0.035 V/\( \mu m \) and 0.2 Hz (Movie 1). As the field is switched on, dense regions emerge in the suspension after an induction time (~90 s) (Fig. 1b). Three-dimensional (3D) clusters form subsequently (Fig. 1c). The resulting clusters are dynamic: they diffuse and merge into larger ones as they meet (Movie 1). However, the growth of clusters is self-limited: as the size of clusters is beyond a critical value, they split into smaller ones (Movie 1). Therefore, the clusters don’t coalesce into a homogenous dense phase but separate from each other with a finite size, giving rise to a discrete dense phase (Fig.1d).

To acquire a full three-dimensional (3D) view of the active clusters, the bacteria are dyed with CFDA-SE (Carboxyfluorescein diacetate succinimidyl ester) (400 nM, Sigma), and the active clusters in the final steady state are scanned by confocal laser scanning microscopy (CLSM). It shows that the active clusters are spherical (Fig. 1e). They suspend in the solvent and are not in contact with the electrodes. Around the dense clusters, a dilute phase of diffusing bacteria can be identified. The dilute phase and the clusters are in a dynamic equilibrium as can be seen in Movie 2. It follows that in the final state, dense clusters are coexisting with a low density phase of bacteria. Therefore, the formation of the dense active clusters is a result of microphase separation, which gives rise to a discrete dense (cluster) phase and a dilute phase. Inside the clusters, bacteria are active and move randomly (Movie 2). No spatial ordering is identified. As we switched off the electric field, bacteria scatter and become uniformly dispersed soon, giving rise to the dissociation of active clusters (Movie 3). It follows that the bacteria in the clusters are biologically active.

In colloidal suspensions which are subjected to an electric field, the electrohydrodynamics (EHD) induces a long-range attraction between charged colloidal particles which stay on the surfaces of electrodes, giving rise to the formation of two-dimensional colloidal crystals\(^{42} \). As the electric field is strong enough to levitate the colloidal particles, three-dimensional (3D) vortex rings forms because of the formation of electroconvection rolls in which the
colloidal particles move and flow along the streamlines. However, in our study, the bacteria are self-propelled and the effect of gravity can be neglected. Therefore, 3D structures can be formed directly without a critical E to balance the gravity. Moreover, the bacteria are active and self-propelled by producing backward flows with flagella. These small-scale fluid flows disturb and distort the convection flows inside the clusters. Therefore, directed motions along the streamlines are not observed in the active bacteria clusters.

The convection flows surrounding the clusters form an external confinement to keep the bacteria together as a cluster (Fig.1f). The stability of the active clusters depends on the competition between the external confinement produced by the flows and the repulsion between the charged bacteria. As the clusters grow and more charged bacteria are included, it becomes increasingly difficult for the clusters to absorb more charged bacteria. Therefore, the growth of active clusters will stop at a critical size. The magnitude of the EHD-induced flows and thus the strength of the confinement on clusters are dependent on the strength of the electric field as well as the frequency f by \( E^2 / f^2 \). For an AEF of a low frequency (<1.0 Hz), the magnitude of the EHD flows can respond and oscillate with the field such that the strength of the confinement on the active clusters experience periodical oscillation, giving rise to the oscillating of clusters. Due to the oscillating, the clusters transform between an expanding state and a shrinking state periodically.

In this study, the radius R of the active clusters in the expanding state is measured to characterize the size of the active clusters. To find out the critical R for splitting, the growth of one cluster is followed from the beginning (Movie 4). The radius R where the splitting occurs is recorded as the critical size \( R_c \). Alternatively, the distribution of R in the final steady states is measured. Figure 2a represents a typical size distribution obtained at \( E = 0.035 \) V/\( \mu \)m and \( f = 0.6 \) Hz (bacteria concentration: 2.4x10^8 cfu/ml). The distribution is characterized by a peak value \( \rho_c \). However, the distribution is not symmetry: there is a tail on the right of \( R_c \). It is found that the maximum size at the end of the tail is highly consistent with the \( R_c \) identified by following the growth process. Based on this result, the maximum sizes in the steady state are taken as the critical size \( R_c \). Observations found that \( R_c \) is sensitive to the frequency of AEF: Increasing the frequency gives rise to the decrease of \( R_c \) (Fig 2b). The mechanism is that increasing frequency leads to the decrease of the magnitude of the EHD-induced flows such that the confinement gets weakened. As a result, the critical size where the repulsive force between bacteria is balanced becomes smaller. In contrast, \( R_c \) does not change significantly as E is tuned (Fig. 2c). Similar observations have been reported in colloidal systems where increasing E does not enhance the aggregation of colloids. The mechanism is that both the EHD flows and the dipolar repulsion between colloidal particles scale as \( E^2 \). Here, we suggest that the weak dependence of \( R_c \) on E originates from the same mechanism.

If the critical size resulting from the balance between fluid flows and the electrostatic repulsion, it is expected that \( R_c \) dose not dependent on the global concentration of the suspension. However, our observations reveal that as the concentration is higher than \( \rho_c = 4.8 \times 10^9 \) cfu/ml, the critical size increases with the global concentration sharply (Fig.2d). Below the concentration \( \rho_c \), the number density of active clusters is low and heterogeneous in the system (left inset in Fig.2d). However, above the concentration of \( \rho_c \), the number density becomes uniform and much higher. Therefore, at high concentrations, the electrostatic repulsion between the active clusters becomes significant such that all clusters are spatially caged by surrounding clusters. The repulsive forces between clusters provide an additional mechanism to balance the internal repulsion between charged bacteria. Based on above understanding, we suggest that the active clusters formed at high concentrations are not equilibrium structures, being distinct from that observed at low concentrations.

For active clusters below the critical size \( R_c \), their radius oscillating with the field at the same frequency (Fig.3a-b, Movie 2). Accompanying the oscillating of R, the concentration of the bacteria inside oscillates between the expanding state and the shrinking state. In the shrinking state, the concentration in the clusters is not uniform: it is characterized by a dense shell (Fig.3b bright region) and a sparse core (Fig.4b dark region). The understanding is that the shrink of the clusters is induced by the increase of the surrounding fluid flows which produce an external pressure on the surface of the clusters. As a result, the condensation starts from the surface and develop toward the core gradually. Nevertheless, accompanying the condensation, the electrostatic repulsions between bacteria increase as well. At the same time, the compression continues only for a finite time (T/4, T is the period of AEF) such that the condensation cannot develop into the core of clusters, giving rise to a dense shell and a sparse core. The core-shell structure produces an interface between a high stress domain (dense shell) and a low stress domain (sparse core). A small density fluctuation on the interface will be amplified by the stress difference and leads to the breaking the core. It follows
that the splitting of clusters arises from the instability of the core-shell structure at the shrinking state. This can be seen clearly from the splitting of forming clusters (Fig.3c-f and Movie 1).

The dependence of $R_s$ on $f$ suggests that the splitting (or merging) of clusters can be triggered (or enhanced) by tuning $f$. To verify this scenario, starting from the steady state at $f = 0.3$ Hz, the frequency is tuned directly to a larger value $f = 0.6$ Hz. As expected, the largest clusters split into smaller ones (Movie 5). By contrast, the decrease of frequency did not lead to the merging of existing clusters. We suggest that as clusters form and reach a steady state, it is difficult for them to perform long-distance diffusion for merging. Therefore, the merging of clusters is dynamically suppressed. This is consistent with the observation that the merging of clusters is frequent in the early stage while in the later stage, splitting becomes relatively more frequent (Movie 1).

Given the concentration of bacteria, the formation of active oscillating clusters occurs only in a finite region in the plane of $E$-$f$. Figure 4a presents the phase diagram measured at the concentration of $2.4 \times 10^8$ cfu/ml. Outside the oscillating cluster region, a stronger electric field leads to the directed motion of bacteria such that the charged bacteria are absorbed on the electrodes and becomes damaged. At high frequencies, it is difficult for the EHD-induced fluid flows to oscillate with field and the long-range attraction between bacteria also becomes weaker. As a result, a condensed phase can form but further condensation for clusters cannot occur. In the condensed phase, it is difficult for both bacteria and the flows to respond synchronically with field. The boundary of condensed phase dense can extend to 100 Hz. The positions of the phase boundaries in $E$-$f$ plane are dependent on the global concentration of bacteria. Increasing the concentration shifts the phase boundaries toward low frequencies (SFig.1a). Increasing concentration also leads to the increase of the peak size and the critical size (SFig.1b). As the concentration is high enough, the clusters become jammed (SFig.1c).

As the strength of fluid flows depends on $E$, the scale of fluid flows is limited by the boundary and the thickness of the chamber. It is expected that increasing the height of the chamber, which promises larger scale of the convection flows, can improve the critical size of the active clusters at the given $E$ and the concentration of bacteria. To verify this scenario, experiments were conducted with two samples with different heights (the distance between the electrodes) at the same $E$ and bacterial concentration. Figure 4b exhibit the size distributions observed. As expected, doubled thickness leads to much more larger sizes of active clusters. The size distribution with doubled thickness shift significantly toward the large size side. Both the peak size and the critical size increase by two times. This result offers a strong evidence that the EHD-induced fluid flows play a critical role in forming the active clusters and confine the motions of bacteria.

**Conclusions**

In summary, charged bacteria in suspension subjected to an AEF exhibit interesting modulated collective behaviors. They form oscillating clusters which oscillate with electric field and have a critical size form splitting. These observations reveal that the collective behaviors of active matters may be tuned and modified by applying control on hydrodynamics with external fields. Clustering has been observed in colloidal systems where the EHD-induced long-range attraction is responsible for the aggregation. We suggest that the aggregation of bacteria is induced by the similar mechanism. The oscillating and shrinking of active clusters produce a high-density shell and a low-density core. The instability of the core-shell structure leads to the splitting as the clusters are beyond a critical size. To have a full understanding on the mechanism of splitting, further observations at single-particle level are critical. Nevertheless, three-dimensional observations on the dynamics of individual bacteria at high concentrations are still a challenge in experiments so far. The formation of dense active clusters of bacterial significantly improves the local concentrations. This has potential applications in clinic applications to detect the bacteria early and quickly.

---

**Fig. 3 Oscillating and splitting.** (a-b) Expanding and shrinking states at $E = 0.035$ V/µm and $f = 0.2$ Hz. (c-f) Formation and splitting of an active cluster. (c) Early stage of the active clusters. (d) Dense shell forms as the concentration in the growing clusters is high. (e) Distorted core-shell structure. (f) Two smaller clusters forms after the splitting. Scale bar: 20 µm.

**Fig. 4 Phase diagram and the effect of height on size distribution.** (a) Different structures that form under an ac field at bacterial concentrations of $2.4 \times 10^8$ cfu/ml. The dashed lines are lines where qualitative changes either in structure or dynamics are observed. (b) Size distribution of clusters formed at different height. $E = 0.035$ V/µm, $f = 0.3$ Hz, $H_1 = 240 \mu$m, $H_2 = 420 \mu$m.
Author Contributions

T. H. Zhang designed the experimental research; MMB and IZI performed experiments and contribute equally; MMB and THZ analyzed experimental data and wrote the paper.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

We thank Wende Tian for interesting discussions. T.H.Z. and K.C. acknowledge financial support of National Natural Science Foundation of China (Grant No. 11974255 and 11635002 to T.H.Z.).

References

1. Colin R, Drescher K, Sourjik V. Chemotactic behaviour of Escherichia coli at high cell density. Nat. Commun. 2019, 10, 5329.

2. Zhang HP, Be’er A, Florin EL, Swinney HL. Collective motion and density fluctuations in bacterial colonies. Proc. Natl. Acad. Sci. U.S.A. 2010, 107, 13626-13630.

3. Makris NC, Ratilal P, Symonds DT, Jagannathan S, Lee S, Nero RW. Fish population and behavior revealed by instantaneous continental shelf-scale imaging. Science 2006, 311, 660-663.

4. Parrish JK, Edelstein-Keshet L. Complexity, pattern, and evolutionary trade-offs in animal aggregation. Science 1999, 284, 99-101.

5. Vicsek T, Czirók A, Ben-Jacob E, Cohen I, Shochet O. Novel Type of Phase-Transition in a System of Self-Driven Particles. Phys. Rev. Lett. 1995, 75, 1226-1229.

6. Gachelin J, Rousselet A, Lindner A, Clement E. Collective motion in an active suspension of Escherichia coli bacteria. New J. Phys. 2014, 16, 025003.

7. Wolgemuth CW. Collective swimming and the dynamics of bacterial turbulence. Biophys. J. 2008, 95, 1564-1574.

8. Lushi E, Wieland H, Goldstein RE. Fluid flows created by swimming bacteria drive self-organization in confined suspensions. Proc. Natl. Acad. Sci. U.S.A. 2014, 111, 9733-9738.

9. Wensink HH, et al. Meso-scale turbulence in living fluids. Proc. Natl. Acad. Sci. U.S.A. 2012, 109, 14308-14313.

10. Wu XL, Libchaber A. Particle diffusion in a quasi-two-dimensional bacterial bath. Phys. Rev. Lett. 2000, 84, 3017-3020.

11. Dombrowski C, Cisneros L, Chatkaew S, Goldstein RE, Kessler JO. Self-concentration and large-scale coherence in bacterial dynamics. Phys. Rev. Lett. 2004, 93, 098103.

12. Guo S, Samanta D, Peng Y, Xu XL, Cheng X. Symmetric shear banding and swarming vortices in bacterial superfluids. Proc. Natl. Acad. Sci. U.S.A. 2018, 115, 7212-7217.

13. Peruani F, Starruss J, Jakovljevic V, Sogaard-Andersen L, Deutsch A, Bar M. Collective Motion and Nonequilibrium Cluster Formation in Colonies of Gliding Bacteria. Phys. Rev. Lett. 2012, 108, 098102.

14. Xu HR, Dauparas J, Das D, Lauga E, Wu YL. Self-organization of swimmers drives long-range fluid transport in bacterial colonies. Nat. Commun. 2019, 10, 1792.

15. Sokolov A, Aranson IS, Kessler JO, Goldstein RE. Concentration dependence of the collective dynamics of swimming bacteria. Phys. Rev. Lett. 2007, 98, 158102.

16. Leptos KC, Guasto JS, Gollub JP, Pesci AL, Goldstein RE. Dynamics of Enhanced Tracer Diffusion in Suspensions of Swimming Eukaryotic Microorganisms. Phys. Rev. Lett. 2009, 103, 198103.

17. Cisneros LH, Cortez R, Dombrowski C, Goldstein RE, Kessler JO. Fluid dynamics of self-propelled microorganisms, from individuals to concentrated populations. Exp. Fluids 2007, 43, 737-753.

18. Ahmadzadegan A, Wang SY, Vlachos PP, Ardekani AM. Hydrodynamic attraction of bacteria to gas and liquid interfaces. Phys. Rev. E 2019, 100, 062605.

19. Belovs M, Cebers A. Hydrodynamics with spin in bacterial suspensions. Phys. Rev. E 2016, 93, 062404.

20. Berke AP, Turner L, Berg HC, Lauga E. Hydrodynamic attraction of swimming microorganisms by surfaces. Phys. Rev. Lett. 2008, 101, 038102.

21. Pierce CJ, Wijesinghe H, Mumper E, Lower BH, Lower SK, Sooryakumar R. Hydrodynamic Interactions, Hidden Order, and Emergent Collective Behavior in an Active Bacterial Suspension. Phys. Rev. Lett. 2018, 121, 188001.

22. Saintillan D, Shelley MJ. Emergence of coherent structures and large-scale flows in motile suspensions. J. R. Soc. Interface 2012, 9, 571-585.

23. Ishikawa T, Sekiya G, Imai Y, Yamaguchi T. Hydrodynamic interactions between two swimming bacteria. Biophys. J. 2007, 93, 2217-2225.

24. Broto T, Bartolo D, Saintillan D. Spontaneous Flows in Suspensions of Active Cyclic Swimmers. J. Nonlinear Sci. 2015, 25, 1125-1139.

25. Sokolov A, Aranson IS. Physical Properties of Collective Motion in Suspensions of Bacteria. Phys. Rev. Lett. 2012, 109, 248109.
26. Wioland H, Lushi E, Goldstein RE. Directed collective motion of bacteria under channel confinement. New J. Phys. 2016, 18, 075002.
27. Pierce CJ, Wijesinghe H, Mumper E, Lower BH, Lower SK, Sooryakumar R. Hydrodynamic Interactions, Hidden Order, and Emergent Collective Behavior in an Active Bacterial Suspension. Phys. Rev. Lett. 2018, 121, 188001.
28. Pierce CJ, et al. Tuning bacterial hydrodynamics with magnetic fields. Phys. Rev. E 2017, 95, 062612.
29. Faire D, Schuler D. Magnetotactic bacteria and magnetosomes. Chem. Rev. 2008, 108, 4875-4898.
30. Vincenti B, Ramos G, Cordero ML, Douarche C, Soto R, Clement E. Magnetotactic bacteria in a droplet self-assemble into a rotary motor. Nat. Commun. 2019, 10, 5082.
31. Meng FL, Matsunaga D, Golestanian R. Clustering of Magnetic Swimmers in a Poiseuille Flow. Phys. Rev. Lett. 2018, 120, 188101.
32. Rajnicek AM, Mccaig CD, Gow NAR. Electric-Fields Induce Curved Growth of Enterobacter-Cloacae, Escherichia-Coli, and Bacillus-Subtilis Cells - Implications for Mechanisms of Galvanotropism and Bacterial-Growth. J. Bacterial. 1994, 176, 702-713.
33. Li J, McLandosborough LA. The effects of the surface charge and hydrophobicity of Escherichia coli on its adhesion to beef muscle. Int. J. Food Microbiol. 1999, 53, 185-193.
34. Minerick AR, Zhou RH, Takhistov P, Chang HC. Manipulation and characterization of red blood cells with alternating current fields in microdevices. Electrophoresis 2003, 24, 3703-3717.
35. Gagnon Z, Gordon J, Sengupta S, Chang HC. Bovine red blood cell starvation age discrimination through a glutaraldehyde-amplified dielectrophoretic approach with buffer selection and membrane cross-linking. Electrophoresis 2008, 29, 2272-2279.
36. Hoettges KF, McDonnell MB, Hughes MP. Use of combined dielectrophoretic/electrohydrodynamic forces for biosensor enhancement. J. Phys. D. Appl. Phys. 2003, 36, L101-L104.
37. Carrigan SD, Scott G, Tabrizian M. Toward resolving the challenges of sepsis diagnosis. Clin. Chem. 2004, 50, 1301-1314.
38. Sikorski RS, Wang L, Markham KA, Rajagopalan PT, Benkovic SJ, Kohen A. Tunneling and coupled motion in the Escherichia coli dihydrofolate reductase catalysis. J. Am. Chem. Soc. 2004, 126, 4778-4779.
39. Hesse WR, Kim MJ. Visualization of flagellar interactions on bacterial carpets. J. Microsc. 2009, 233, 302-308.
40. Berg HC, Anderson RA. Bacteria Swim by Rotating Their Flagellar Filaments. Nature 1973, 245, 380-382.
Supplementary materials

SFig.1 Clusters formed at high bacterial density. (a) Phase diagram at bacterial concentration of 6.0x10^8 cfu/ml. (b) Size distribution graph of clusters. \( E = 0.03 \) V/\( \mu m \), \( f = 0.45 \) Hz, \( H = 200 \) \( \mu m \). (c) Under higher bacterial density, clusters are larger and more dense. Space bar=50 \( \mu m \).

Description of movies

**Movie 1.** Formation of clusters at \( E=0.035 \) V/\( \mu m \), \( f=0.2 \) Hz. The field of view is 124 x 93 \( \mu m^2 \). The rate of movie is 10 frames per second. The movie runs for 210 s of real time.

**Movie 2.** Dynamic equilibrium can be seen at \( E=0.035 \) V/\( \mu m \), \( f=0.45 \) Hz. The field of view is 96 x 72 \( \mu m^2 \). The rate of movie is 10 frames per second. The movie runs for 4.4 s of real time.

**Movie 3.** Active clusters disperse at \( E=0.035 \) V/\( \mu m \), \( f=0.6 \) Hz. The field of view is 96 x 72 \( \mu m^2 \). The rate of movie is 10 frames per second. The movie runs for 43 s of real time.

**Movie 4.** One cluster grows from the beginning at \( E=0.035 \) V/\( \mu m \), \( f=0.5 \) Hz. The field of view is 120 x 90 \( \mu m^2 \). The rate of movie is 10 frames per second. The movie runs for 270 s of real time.

**Movie 5.** Large clusters split into smaller ones when frequency is tuned from 0.3 Hz directly to 0.6 Hz, \( E=0.035 \) V/\( \mu m \). The field of view is 116 x 87 \( \mu m^2 \). The rate of movie is 10 frames per second. The movie runs for 375 s of real time.